

INFORMATION FOR CONTRIBUTORS

GENERAL

All manuscripts should be addressed to the Editor, *Current Science*, P. B. No. 80001, C. V. Raman Avenue, Bangalore 560 080. Submission of an article will be held to imply that it has not been previously published and is not under consideration for publication elsewhere, and further, that if accepted, it will not be published elsewhere. *Three copies of contributions of all categories* are required, with a letter of transmittal giving (i) names and complete addresses of the authors and (ii) title of the contribution and the category in which it is submitted (see below).

Current Science is a multidisciplinary journal and therefore research and review papers of general significance that are written clearly and well organized will be given preference. All papers will be first assessed by a Reviewing Editor. Papers found unsuitable in terms of the overall requirements of the journal will be returned to the authors. The others will be sent for detailed review. *Both solicited and unsolicited material will be reviewed.* Authors of these papers will be notified of acceptance, rejection, or need for revision of the paper. Returned papers cannot be resubmitted. Illustrations and other material to be reproduced from other publications must be properly credited; it is the authors' responsibility to obtain permission for reproduction (copies of letters of permission should be sent).

CATEGORIES OF MANUSCRIPT

General articles (not exceeding 5000 words) discuss current trends in research in a field that will be of interest to readers outside the field, interdisciplinary topics, science policy, and administration, or some aspect of the application of science and technology to human needs or the impact of science and technology on society/ecosystems/life. They should include a summary not exceeding 100 words, introductory paragraph(s), brief subheads at appropriate places to point to what follows, illustrations that will help a general reader, and references.

Review articles (not exceeding 5000 words) are expected to survey and discuss recent developments in a field. They should be well focused and organized, and avoid a general, 'textbook' style.

Research articles (not exceeding 4000 words) should report research results of fairly major significance. They should include an abstract not exceeding 100 words, introductory paragraph(s), and brief subheads.

Research communications (not exceeding 2000 words) should contain important findings that are novel and of fairly broad interest. They should include a brief abstract and an introductory paragraph. Text should not be broken up under subheads.

Correspondence includes letters, not exceeding 500 words, that are of general interest to scientists. All letters cannot be published.

Scientific correspondence contains technical comments, including those on articles or communications published in *Current Science* within the previous six months. Letters may be reviewed and edited.

Research news articles are intended to inform nonspecialists about recently published advances or important findings discussed at a meeting. Authors should also send a copy of the paper(s) on which the article is based. Meeting reports should avoid merely listing brief accounts of topics discussed, and must convey to readers the significance of an important advance.

Research accounts articles are intended to be personalized reviews of research from the authors' own laboratory, based on a body of published work. The articles must provide appropriate background to the area in a concise introduction, which should also serve to place the author's work in proper perspective. Articles will normally

not exceed 8 to 10 printed pages.

Opinion articles present views on issues related to science and scientific activity. **Commentary** articles should contain expository notes on issues related to science and scientific activity.

Book reviews. Unsolicited reviews will also be considered. Reviews that merely 'list' brief descriptions of the contents cannot be published. Reviews should have 'context' and convey some information about the subject of the book.

Historical commentary and notes inform readers about interesting aspects of personalities or institutions of science or about watershed events in the history/development of science; most are expected to relate to India. Illustrations are welcome. Brief items will also be considered.

MANUSCRIPT PREPARATION

Manuscripts should be typed double-spaced on one side of white bond paper (21 × 28 cm). The pages should be numbered consecutively, starting with the title page and through the text, reference list, tables and figure legends. The title should be brief, specific, and amenable to indexing. Not more than five **keywords** should be indicated separately; these should be chosen carefully and must not be phrases of several words. **Summary** and **abstract** should not have more than 100 words and should convey the main point of the paper, outline the results and conclusions, and explain the significance of the results.

Text. All papers should have a brief introduction. The text should be intelligible to readers in different disciplines and technical terms should be defined. Tables and figures should be referred to in numerical order. All **symbols** and **abbreviations** must be defined, and used only when absolutely necessary. Superscripts and subscripts and ambiguous characters should be clearly indicated. **Units of measure** should be metric or, preferably, SI. Methods should, as far as possible, be described briefly in appropriate table and figure legends.

Figures. In the case of line drawings one set of originals (without any lettering) is sufficient, with two copies containing lettering. In the case of photographs good prints are required with each copy of the manuscript; photocopies are not acceptable. Line drawings should be roughly twice the final printed size. The correct orientation should be indicated if not clear.

Photomicrographs and other photographs that require it must have a scale bar, which should be defined clearly in the legend. Primary data should be submitted as far as possible (e.g. actual photographs of electrophoretic gels rather than idealized diagrams).

References should be numbered in the order in which they appear, first through the text and then through table and figure legends. The following are examples of ways of writing references:

1. Mukundan, T. and Kishore, K., *Curr. Sci.*, 1991, **60**, 355-362.
2. Constantine, G., in *Biology of Bats* (ed. Wimsatt, W. A.), Academic Press, New York, 1970, vol. 1, pp. 319-322.

Acknowledgements should be brief. Footnotes are not allowed except to identify the corresponding author if not the first.

Cover photographs. Good photographs (colour or black and white) that pertain to a submitted paper will be considered for use on the cover. Good *prints* and a legend should be submitted with the manuscript. In the case of a colour picture, a transparency will be required for printing if accepted.

PROOFS AND PUBLICATION

Two sets of galley proofs are sent to the corresponding author. A reprint order form accompanies the proofs.

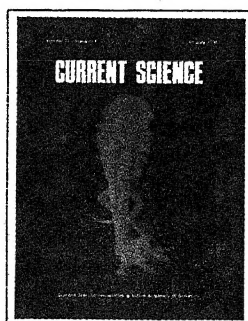
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COVER. Limbs developed at the tail tip of *Microhyla ornata* tadpole, after treatment with vitamin A solution. [photo: S. K. Dutta]. See page 61.

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In this issue

The language of DNA: How to understand what a sequence tells you about itself

Reduced to its elementary building blocks, a piece of English text is just a 'character string' – a chain of 50-odd symbols (alphabets, digits, punctuation marks, etc.) arranged in a sequence, some symbols occurring more often than others. A sonnet by Shakespeare, a railway timetable, a common minimum programme of the government, announcements of the Nobel prizes and editorials in *Current Science* are all character strings. However, despite their widely differing contents and styles, even a casual look at them is enough to make out what they tell you.

DNA, the hereditary material, is also a collection of (simpler) character strings, made up of just the four symbols A, T, G and C. The DNA sequences also differ greatly in their 'messages'; some 'code' for proteins, others for nucleic acids, still others promote the manufacture of their own copies, while yet another set prevents the copying process. Unlike English prose, however, a casual (or even a thoroughly and painstakingly non-casual) glance is *not* enough to tell us as to which of these (or other) categories does a given DNA sequence belong. And we do need such information, the need becoming more and more pressing as the days go by. The charge of the light brigade on human and other genomes continues to generate DNA sequences at increasingly faster rates. Ironically, almost 90% of the DNA, at least in higher organisms, performs no *known* function (and is therefore crudely called 'junk DNA'). To put to use the sequence data, obtained at enormous expense, it is

essential to have the means to identify whether a sequence at hand is junk or functional, and if functional, to guess its likely role.

S. Tiwari *et al.* review (page 12) the different methods used for making sense of DNA sequences. In principle, the approaches are no different from what we use (mostly subconsciously) to distinguish between, say, Maugham and Hemingway; most authors have a characteristic 'style' which we learn to recognize after reading them for a while. The DNA sequences (coding regions, promoters, enhancers and the like) too have their characteristic 'signatures', which can become apparent if we have a number of examples of each of the categories. However, these are not readily apparent to the human senses, (well, not quite; there are reports of DNA sequences being set to music and the connoisseurs claiming to be able to distinguish between a Beethoven-like protein region from the cacophony of a noncoding one!), and computers have to be brought in. A variety of mathematical and statistical techniques have to be used to make this process of pattern-recognition automated, objective and rigorous. Ramaswamy and colleagues describe many of these (including the ones developed by them) in detail, and also point out the criteria (sensitivity, specificity, etc.) of judging the relative merits of these approaches. Like all vibrantly active fields, there has been considerable progress in the recent past, and there is considerable scope for more improvement. This is one (and perhaps the only) area of modern molecular biology where the physicists/mathematicians/computer scientists, with their (indisputably) infinite ignorance and (arguably) unlimited intelligence,

may manage to make really important contributions.

N. V. Joshi

Seeing is believing: Examining the dinucleotide frequencies in a DNA sequence

The simplest descriptors of the composition of a DNA sequence are the proportions of the four bases A, T, G and C. Logically, the next set of descriptors are the proportions of the sixteen possible dinucleotide pairs; and sequences having the same composition of the four bases can differ markedly from each other when the sixteen proportions are compared. Knowing the extent and nature of such differences is of interest to the sequence analysts. The self-evident and simplest way of doing it is to compare the sixteen proportions of the first sequence with the corresponding ones of the second – sixteen pairs of thirty-two numbers. This, however, poses a major problem. Most biologists (from the lowly taxonomists to the super-elite biotechnologists) are extremely reluctant to reduce their finding to a mere set of numbers, and even more reluctant to look at others' findings when expressed as numbers.

If all you want to do is to describe, then a picture can do the job much better – something well known for centuries. To illustrate with an unrelated example, one can more easily see the differences in the pattern of variation of rainfall throughout the year between say Calcutta and Bangalore, by looking at their mean monthly rainfall profiles. An ingen-

ious variation of this theme is adopted by A. Pan *et al.* (page 50). Instead of the usual *X* and *Y*-axes in a plane, they use sixteen axes, represented as arrows beginning at the origin, with an angle of 360/16 degrees between the neighbouring arrows. These axes now represent the sixteen nucleotide pairs, and the proportion (of AA, AT, ..., etc.) found in any sequence can be marked off along the axis (arrow) corresponding to that nucleotide pair. A closed sixteen-sided polygon, formed by successively joining the points marked on adjacent axes, (or a 'map', which the authors mystifyingly call a contour diagram) shows all the sixteen proportions simultaneously. Two or more different sequences can now be compared with just a single glance at the colourful, glowing com-

puter screen. The shapes of these polygons display the same information as contained in the sixteen pairs of numbers, but the eye, evolved over millions of years for instantly interpreting complex patterns, seems to be able to extract some meaning out of it.

It has not escaped the authors' attention that this could be generalized to examine trinucleotide frequencies and similar other features. They are also aware of the large number of different ways in which the sixteen dinucleotides could be assigned to the sixteen directions, and after some experimentation, have settled on one which gives aesthetically more appealing patterns. They do not mention a linear diagram, however, and it is entirely possible that someone else would, justifiably

claiming that such a subtle change produces profound differences in the displayed patterns.

After the descriptions, follow the inferences, and the authors describe how the maps for plant, parasite and random sequences show *significantly* different dinucleotide proportions. When you have pretty pictures to convince you, why worry about such mundane technicalities as statistical tests? The appealing combination of molecular biology and computer graphics is far more persuasive than a feeble statistical phrase like 'significantly different, $p < 0.05$ '. After all, it is the performance of the advertising and publicity section, and *not* statistical quality control, which make or break a company.

N. V. Joshi

INDIAN INSTITUTE OF SCIENCE

BANGALORE 560 012

Applications are invited from Indian nationals preferably below the age of 35 years for faculty positions at the level of Assistant Professor in the Department of Biochemistry. The candidates if selected are expected to develop and maintain independent research in any chosen area of biochemistry as well as collaborate with other faculty and contribute to teaching programme.

The candidates should have a Ph.D degree with about 3 years of postdoctoral research experience. Those who wish to develop new programmes of research in areas other than those in which they are presently working, or whose interests bridge basic and applied research are also encouraged to apply. Small start-up funds could be provided. The total emoluments at the minimum of the scale (Rs 3700-125-4950-150-5700) are around Rs 1,23,000 per annum. Interested persons should send: (1) curriculum vitae, list of publications, important reprints and name and address of three referees and (2) a brief description of the proposed research programme and the minimum facilities required for carrying it out, to Prof. M. Vijayan, Chairman, Division of Biological Sciences, Indian Institute of Science, Bangalore 560 012, India, within two months of the appearance of this advertisement. The referees may be requested to send their assessment directly to Prof. Vijayan.

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REGISTRAR

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CORRESPONDENCE

Herbal medicines

In a letter published recently in *Current Science* Tripathi¹ has stressed on the importance of traditional Indian medicines. In the present scenario where 80% of the world population has no access to the benefits of western medicines due to financial constraints, it is hardly necessary to emphasize the relevance of traditional remedies which constitute a major part of the health care system in the developing countries and are also entering the therapeutics in the developed countries. However, most of these drugs are derived from plant products, and a couple of discomfoting reports published recently reveal some problems associated with the use of herbal medicines (HM).

In most of the cases HM escape toxicity testing before they are marketed. Some of them contain mercury, lead, arsenic and poisonous organic substances in harmful amounts. Hepatic failure and even death following ingestion of HM have been reported². A perspective study shows that 25% of the corneal ulcers in Tanzania and 26% of the childhood blindness in Nigeria and Malawi were associated with the use of traditional eye medicines³.

Quantitative variation of the constituents may be very high from batch to batch of a plant material. For example,

paeoniflorin content of 12 samples of red peony root (*Paeonia lactiflora*, used as a traditional Chinese remedy for eczema) bought in London was found⁴ to range from 0.01% to 4.5%. This makes correct dosing of HM a difficult task.

Sometimes patients use traditional and western medicines simultaneously. The interaction of these two types of drugs *in vivo* may be dangerous. Piperine present in many Ayurvedic formulations is known to increase the toxicity of theophylline, a bronchodilator routinely used by the asthmatics and also of phenytoin, an anticonvulsant⁵.

Alarminglly HM, in some cases, are found to be admixed with allopathic medicines. In Leicester Royal Infirmary one sample of traditional Chinese medicine, given to a lady for eczema, was found to contain a steroid⁶. Several undeclared drugs including phenylbutazone, diazepam and corticosteroids were detected in a traditional Chinese cure for arthritis⁷.

Safe use of HM is a burning question at present. A project is under way at the WHO Collaborating Centre for International Drug Monitoring in Sweden to classify common toxic ingredients of HM (ref. 8). One government-funded agency in the UK and another in the US have

been appointed to investigate complaints from the consumers and to recommend removal of the harmful products from the market. With the increase in the popularity of HM throughout the world, it is necessary to ensure safety of the patients.

1. Tripathi, Y. B., *Curr. Sci.*, 1996, 70, 416.
2. Dickens, P., Tai, Y. T., But, P. P., Tomlinson, B., Ng, H. K. and Yan, K. W., *Forensic Sci. Int.*, 1994, 67, 55-58.
3. Harries, A. D. and Cullinan, T., *The Lancet*, 1994, 344, 1588.
4. Harper, J., *Br. Med. J.*, 1994, 308, 489.
5. Sears, C., *New Sci.*, 4 November 1995, 148, 37-40.
6. Graham-Brown, R. A. C., Bourke, J. F. and Bumphrey, G., *Br. Med. J.*, 1994, 308, 473.
7. Stricht, B. I. V., Parvais, O. E., Vanhaelen-Fastre, R. J. and Vanhaelen, M. H., *Br. Med. J.*, 1994, 308, 1162.
8. Edwards, R., *Br. Med. J.*, 1995, 311, 1569-1570.

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Global warming and forest fires in tropical Himalaya

This is in reference to the article 'Ecology of forest fires in chir pine (*Pinus roxburghii* Sarg.) forests of Garhwal Himalaya' (R. M. Semwal and J. P. Mehta, *Curr. Sci.*, 1996, 70, 426-427).

P. roxburghii indeed is one of the dominant tree species of tropical Himalaya¹. In general, tropical forests produce nearly two and ten times litter biomass compared to that in temperate and arctic

climates, respectively and are thought to have proportionate decomposition capacities under adequate moisture conditions². Higher temperature tends to deplete top soil moisture at a faster rate, and if not

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duly replenished, can restrict microbial activity and cause crisp dryness of litter biomass under low-humid conditions favouring forest fires.

In pine forests, needles decompose slowly due to its higher lignin content. Lignin resists microbial attack³ and is reported to accumulate in the litter, exerting a negative effect on its further degradation⁴. The leaf biomass therefore accumulates in pine forest.

The fires of summer 1995 in pine forests in western and central Himalayas is typical in enormity of magnitude and the extensive floral and avi-faunal losses it caused. The fire was reported along the broad distribution range of *P. roxburghii* from almost every district in Himachal Pradesh and various locations extending up to Garhwal Himalaya. In Himachal Pradesh, the Dharmshala Circle alone reported 226 forest fires between May to June⁵. The summer also recorded prolonged dry spell and a mean temperature rise of the order of 2–3°C above normal⁶.

The anomaly of temperature rise of 2–4°C has been widely discussed under the proposed climatic change, leading to

global warming by middle of next century⁷. Drought is one of the chief fears anticipated⁷, implying that vulnerability of pine forest to seasonal fire may increase under the situation.

1. Singh, J. S. and Singh, S. P., *Bot. Rev.*, 1987, 53, 80–192.
2. Deighton, J., *Can. J. Bot.*, (Suppl. 1), 1995, 73, 1349–1350.
3. Ried, I. D., *Can. J. Bot.*, (Suppl. 1), 1995, 73, 1011–1018.
4. Johansson, M. B., Berg, B. and Meentemeyer, V., *Can. J. Bot.*, (Suppl. 1), 1995, 73, 1011–1018.
5. Anon., *Indian Express*, 1995, 11 June 1995.
6. Anon., *Indian Express*, 1995, 6 June 1995.
7. Mansfield, T. A., *Prof. Hortic.*, 1991, 5, 3–9.

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R. L. Semwal adds:

Our studies on chir pine forests and

associated grazing lands in Garhwal Himalaya deal with the effects of forest fire on soil, phytosociology, biomass and productivity, litterfall and litter decomposition and nutrient return through litter. Based on our work and available published literature from this region, presently we are not in a position to establish any precise relationship between forest fires and global warming phenomenon. However, as has been pointed out by Vats, it is true that warming of local atmosphere takes place during severe forest fires. Thus, in-depth studies need to be conducted in this direction to explore any such relationship. Nevertheless, we ought to have a well-thought programme to fight with the wild fires in Himalayan region which incurs environmental and social losses on an unmeasurable scale.

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RESEARCH NEWS

Classifying the kiwi

Kartik Shanker

Molecular genetics has, in recent years, become an invaluable tool for conservation biologists. Present thinking^{1,2} suggests that the higher the genetic variability within a species, the better are its chances for survival, and most work to date has centred around this. There has been substantial debate^{1–5} about the importance of genetics in conservation, but many ecologists⁶ still believe that population studies or behavioural studies are more useful. In the light of recent work^{2,7}, however, it seems clear enough that molecular genetics is here to stay in conservation. In a world with limited resources and an overloaded conservation schedule, tough choices have to be made as to which species, and specifically which populations, are to be conserved. These choices

would depend on our priorities and current opinion favours retention of genetic diversity^{1–5}. If we do assign priorities based on genetic diversity, we obviously need detailed studies in molecular genetics.

Baker and co-workers⁷ studied the molecular genetics of brown kiwis in New Zealand. The work is noteworthy for two reasons; firstly, they found levels of genetic differentiation between populations that had not been found in bird populations earlier. Secondly, they showed that the taxonomy of the brown kiwi needed to be reviewed and perhaps changed in the light of their study. This is important because the kiwi is an endangered bird and conservation priorities do need to be assigned.

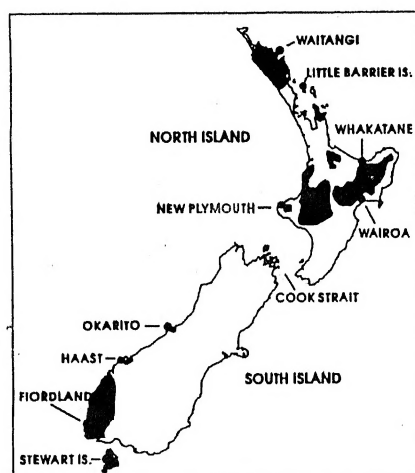
Molecular genetics can help clarify taxonomic errors because traditional taxonomies depend on morphological characters which may not reflect true genetic distances between species². Since most work has depended on the subjective classification of taxonomists, it is likely to be riddled with errors. The case of the Camden County gopher clearly illustrates this².

In 1898, a distinct species of gopher was described in Camden County, Georgia, USA as *Geomys colonus*. The population consisted of very few individuals and was listed as endangered. However, a recent molecular genetic survey showed that this species was not genetically distinct from the more common *Geomys pinetis*, which is found all over USA. In

Table 1. Morphological characters used to classify the kiwi and the results of the allozyme and mtDNA work (from Baker *et al.*, 1995)

Character	Population					
	North Island	Okarito	Haast	Fiordland	Stewart Island	Spotted kiwis
Morphology						
Plumage colour	Brown	Grey	Rufous	Dark grey	Dark brown	Spotted fawn
Feather tips	Stiff	Soft	Soft	Soft	Soft	Soft
Large tarsal scutes	17	7	4	5	6	6
Facial bristles	Long	Short	Short	Short	Short	Short
Feather lice						
<i>Apterygon</i>	<i>A. mirum</i> ; <i>A. rodericki</i> on Little Barrier Island	New species	Absent	<i>A. dumosum</i>	<i>A. dumosum</i>	<i>A. hintoni</i> *
<i>Rallicola</i>	Absent?	<i>R. gadowi</i>	<i>R. gadowi</i>	<i>R. gadowi</i>	<i>R. gadowi</i>	<i>R. pilgrimi</i> † and <i>R. gracilentus</i> *
Allozymes and mtDNA						
AK-1	A > 0.9; C absent	A = 0.95; C = 0.05	B = 0.17; C = 0.83	A = 1.0	A = 1.0	A = 0.98
Hb-2	A = 1.0	A = 1.0	B = 1.0	B = 1.0	B = 1.0	B = 1.0
Ldh-2	A > 0.8; B and C rare	A = 0.4; C = 0.6	A = 1.0	A = 1.0	A = 1.0	A = 1.0
Cytochrome b†	2–11	12 and 13	14	15 and 16	17–22	1 and 23

*Found only on little spotted kiwi; †Found only on great spotted kiwi.; ‡Numbers refer to haplotypes listed in Figure 2.

**Figure 1.** Distribution of the various populations of brown kiwis in New Zealand (from Baker *et al.*, 1995).

fact, there were wider genetic differences across different populations of *G. pinetis*. Hence, *G. colonus* did not warrant recognition as a separate species. Either a mistake had been made in the original classification or *G. colonus* had gone extinct and was replaced by the common *G. pinetis*. In either case, incorrect taxonomy led to an inappropriate conservation priority for the Camden County population of gophers.

Kiwis are small, non-flying birds, found only in New Zealand. Unfortunately, kiwi populations have been fragmented by

hunting, habitat destruction and introduced mammalian predators. They are now found in small, discrete populations that are unlikely to exchange genes. Brown kiwis are found on North Island, South Island and the smaller Stewart Island (south of South Island) (Figure 1). Initially, the North and South Island populations were recognized as separate species (*Apteryx mantelli* and *Apteryx australis*, respectively) on the basis of morphological characters. Subsequently, they were clubbed as a single species and accorded the status of subspecies (*A. australis mantelli* and *A. australis australis*). The Stewart Island population was also recognized as a subspecies (*A. australis lawryi*). Although this classification was widely accepted, reports of differences in feather lice, blood proteins and possibly calls shed some doubt on its validity (for a review, see ref. 7 and references therein). Baker *et al.*⁷ attempted to resolve the issue using molecular genetic techniques.

A number of studies in recent times have been done on maternally inherited mitochondrial DNA, because they provide an excellent source of genetic markers and can be used to reconstruct intra-specific phylogenies of matriarchal lineages⁸. Baker *et al.*⁷ examined differences in the cytochrome b gene from mitochondrial DNA. In the 60 kiwis studied, 654 bp of mtDNA was sequenced; there were 21 types of sequences due to variation at 42 sites. Based on these 21 types, the brown kiwis could be separated into five

populations – the North Island population, the Stewart Island population and three different populations on South Island. Remarkably, none of the populations shared any sequence type.

As one might expect, the North and the South Island birds were clearly divided. However, the northernmost South Island population (the Okarito population) turned out to be a sister group of the North Island population. Baker *et al.*⁷ suggest that the two sub species can now be reinstated as separate species. This is based on the result that the genetic distance between the two populations is as great as that between the two species of spotted kiwi (*A. haastii* and *A. oweni*) (Figure 2). Also, the Okarito population of the South Island can be classified as the same species as the North Island population.

The most remarkable aspect of their result, however, was the extreme structuring of the matrilineal lineages of cytochrome b, with virtually every population showing private alleles (Table 1). This is a first for mtDNA studies in vertebrates. Baker *et al.* also looked at genetic differences between populations by studying the variation in enzymes. The results from the allozyme study confirmed the results of the mtDNA work, showing levels of subdivision seen only in vertebrates such as salamanders, cave-dwelling fishes and small mammals, all animals with very poor dispersal power and disjunct populations.

Unlike small mammals, bird populations were not expected to be genetically

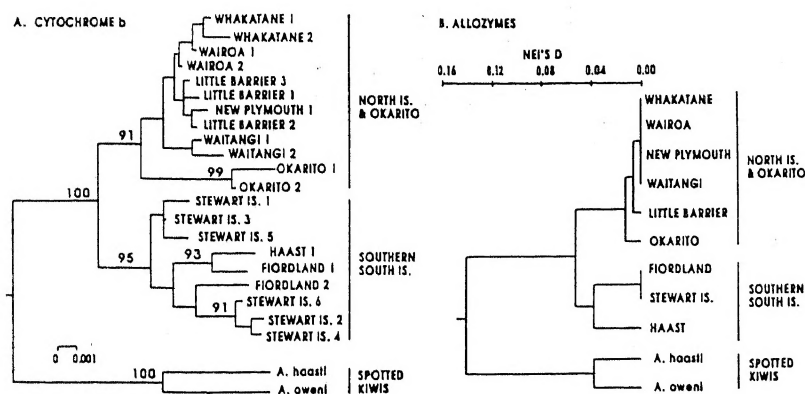


Figure 2. The mtDNA and allozyme trees constructed for the kiwi populations (from Baker *et al.*, 1995).

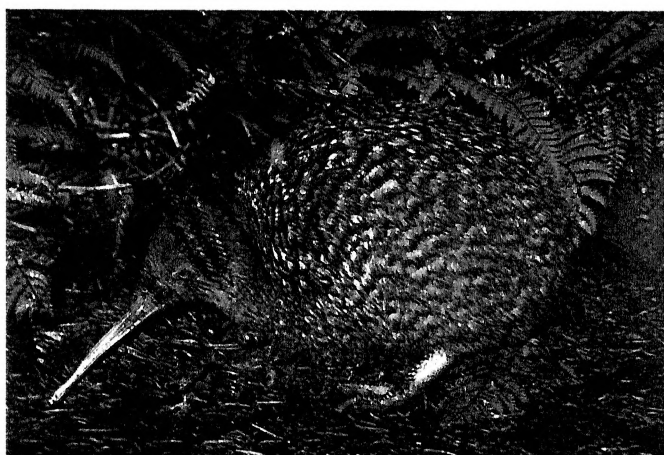


Figure 3. A great spotted kiwi (*Apteryx oweni*); the brown kiwi (*Apteryx australis*) is a little smaller and its body colour is a streaked dark brown and light brown. [The *Encyclopedia of Birds* (eds Perrins, C. M. and Middleton, A. L. A.), George Allen and Unwin, London, 1985.]

different from each other. Their ability to disperse (due to flight) would result in greater exchange of genes between populations and less genetic difference between populations⁹.

Kiwis, however, seem to be more like small mammals than birds (Figure 3). They are the smallest of the seven families of ratite birds. Ratites are non-flying birds that lack the keeled breastbone to which the pectoral muscles are attached. Kiwis, like other ratites, have an adult plumage that is the same in structure as the juvenile down of other birds. They live in burrows, and are essentially nocturnal. These and

other features such as hair-like feathers, facial bristles, two functional ovaries, a well-developed sense of smell and absence of flight make them more like small mammals in their ecology¹⁰.

The brown kiwis were once widely distributed in New Zealand. Range fragmentation must have accelerated since the arrival of humans ca 1000 years ago, due to both loss of habitat and hunting. A simple biogeographical hypothesis suggests that the southern South Island population is a remnant of the original population. This then colonised northward and diverged⁷.

The kiwis are an ancient lineage, judging from Gondwanaland distributions, and have had 40–80 million years to evolve in the isolation of New Zealand. Baker *et al.* used the RFLP (restriction fragment length polymorphism) clock of 2% sequence divergence per 1 million years to estimate divergence in New Zealand. They estimate that the basal population diverged from the Okarito population 900,000 years ago. The divergence between the Okarito and North Island populations would have occurred 500,000 years ago and that within the North Island populations, 200,000 years ago.

To summarize, the truly remarkable finding of this work is the fact that each population has private alleles for cytochrome b. The value of their work lies in their re-classification of the brown kiwi, which sheds new light on its status and makes it necessary to reevaluate conservation priorities. This work emphasizes the role of molecular genetics in conservation biology and stresses the need to continuously assess and review conservation practices. Certainly various other factors such as population ecology, behaviour and even aesthetic value may need to be taken into consideration, but this should in no way detract from the role that molecular genetics has in the conservation biology of tomorrow.

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Patenting and commercializing biotechnology inventions

Suresh Chandran

To someone with a viable invention and no production resources or finance, a patent can give options otherwise not possible. Patents attract capital, help source research funds and provide licensing opportunities. Yet patenting is an expensive business. Even though, filing a complete specification in India may cost only Rs 200, the attorneys' fee may amount to hundred times that figure! Since most therapeutic, diagnostic or food product inventions have no prospect of grant in India and since the market prospects are often better in another country, it makes commercial sense to secure patents in foreign countries. Even once a patent is granted, costs continue. Maintenance, renewals, defence and active exploitation of the rights, monitoring competitor activity, etc. will follow. The costs of pursuing patent protection must therefore be balanced against the holding of granted patents or pending patent applications.

Some pointers can prove helpful. One can begin by asking: Is the invention really *new*? Is it commercially attractive? Is there a market need for the invention or must it be developed? Is the invention a marked improvement over currently available solutions? Is the merit easily demonstrable? A great majority of patents are commercial flops. If the commercial value of the invention is low, patenting efforts should be abandoned. Also if the invention is in a 'hot' area, the likelihood of the technology advancing before the invention is granted a patent is very real. The cost may not justify patenting if protection is short-term. Another vital decision is at what point in time should the inventor initiate filing procedures without compromising the validity or the scope of the ensuing patent at a later date? Mostly these are business decisions and can benefit from the informed opinion of patent attorneys. There is also the folly of rushing too early to file a patent. Technology takes time to mature for markets. If one is not careful, by the time the technology is ready for commercial exploitation, its patent-life would have run out. This is more so for biotechnology,

where regulatory approval takes several years of the patent life.

Of the various intellectual properties, utility patents and trade secrets are the most important in biotechnology. Several kinds of biotechnology-related products can be patented. These include: transformed cells, isolated proteins, mutant proteins and active-site portions of antibodies (*Cellular and molecular biology*); transgenic plants and animals (*Genetic engineering*); methods for preparing MoAbs, MoAbs with various specificities against various microbial antigens, hormones, endotoxins, etc. (*Hybridomas*); EIAs, labelled antibodies for scanning (*Diagnostics*); recombinant vaccines, antibiotics, therapeutic peptides, hormones etc. (*Biologicals, therapeutics*). Use claims can be handy when the processes are well known, and only say the microbe varies. All these inventions besides being eligible subject matter of patents, should comply with the conditions of novelty, utility, non-obviousness and enablement.

Using patents and trade-secrets simultaneously

While the trade-secret protects the invention by withholding information, the patent protects by disclosing information. Indeed, a trade-secret is an ideal form of protection as it does not cost anything and lasts forever, at least until it is discovered by reverse engineering. Trade secrets can be licensed out but prior to doing so, it is critical to enter into a confidentiality agreement with the potential licensee. Employees who must have access to such trade secret information must have express confidentiality obligations and be schooled against inadvertent disclosure.

Biological material can be replicated in large quantities from minute amounts of the starting material, be it clones, cell lines, gene sequences, etc. Loss of proprietary control over the material can mean serious consequences to the owner of the material and therefore it is increasingly important to institute agreements by resident as well as visiting

scientists on the use of proprietary biological material. Cases of lost clones and cell-lines from publicly funded laboratories finding their way into private hands are not unheard of.

The hybridoma for the generation of specific monoclonal antibody against a specific antigen is often kept as know-how. The microbial strain disclosed in a patent and commercially exploited need not be the same, as improved strains are employed in fermentation. Media composition, culture conditions, isolation and purification processes for products can be maintained as know-how. Clinical data or toxicology data may also be withheld as know-how. Even when a patent application is to be filed, there may be ancillary technologies that need not figure in the application but are important to make the product that embodies the patented invention. These are better preserved as trade secrets than as patents. It may be possible to practise both patents and trade-secrets together and it is advisable to disclose only the minimal information mandated by the law for grant of a patent.

Licensing mechanisms

Where funds are scarce as in universities and most publicly funded research institutes, the decision to file a patent is itself risky. The obvious strategy then is to find a licensee who will bear or at least share patenting cost. Intellectual property administration and technology licensing are discreet but inter-related activities. New patterns of technology licensing relationships are emerging and these range from ordinary licenses, marketing partnerships to joint ventures. Research institutions that are publicly funded generally have primary commitment to public good followed by creation of knowledge. Pursuing these goals and simultaneously making some money off the research requires adequate policy decisions at the administrative level because technology transfer activities do affect the freedom of the faculty to the choice of the research project, the right to publish where and

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when and also to the choice of collaborators. With biotechnology inventions in general, commercialization is capital-intensive. It is advisable for small research institutions to make a deal of some sort with a larger company which has the requisite resources for successful commercialization.

Licensing

Exclusive, semi-exclusive or non-exclusive licence may be opted. Licences may also be granted on the basis of territory or field of application. The stage of development of the technology, market size and the level of protection available influence licensing activity. The strength of the patent is a major factor in licensing negotiations. However, it is unwise to put away negotiations till grant of a patent as this would mean throwing away precious time for product-development and blunt a major competitive advantage. Market research, technology evaluation and patent search should precede technology transfer and licensing. The act of selecting the *right* licensee who is capable of developing and commercializing the invention needs to be repeatedly emphasized. Some traits one should be looking for in licensees include: (i) the capability for absorption of the invention and its adaptation to field use, (ii) financial strengths in case regulatory approval and development for the product is envisaged, and (iii) the motivation of the company to commercialize the invention. Often technologies are bought by companies to stifle competition to their own products. It is therefore desirable that one looks up the company's product line and ensure that there is no clash of interest. Some of this information can be obtained from the annual reports of the potential licensees, their business plans, the range of products they intend to bring out and the markets they compete in, etc. These can help compare potential candidates for negotiating a licence. It is almost improbable to find the 'ideal' licensee and compromises have to be made. The

diverse objectives of the partners makes for a conflict of interest situation. Contentious issues often pertain to the:

Scopes of the licence; Value of the technology—quantum and timing of payments; Determination of milestones, terms of intervention; Indemnity and insurance, e.g. where biologicals for human use are involved.

A win-win attitude from the beginning of the partnership is essential. It should be realized that only when the invention is successfully commercialized, royalties would accrue. Industries must also on their part appreciate the limitations of research institutes and not view licensing negotiations as a one-time opportunity to seize advantage. Two related issues from experience, which in retrospect are very critical to successful technology transfer are, the incorporation in the licence document of a fool-proof reporting system to document the licensee's efforts at commercialization. This would aid the licensor initiate intervention procedures if progress is wanting. The second issue concerns ensuring meticulous royalty/sales record keeping. This will facilitate access of the licensor to audited royalty payment records. It must also be settled as to who in the partnership would bear the costs of infringement litigations if any, the company, the institute or jointly. Retaining know-how helps salvage the invention in case somebody succeeds in working around the patent. Technology-pricing is also tricky. If the technology will have a short life, it is a correct strategy to negotiate for substantial lumpsum payments in the first years. Companies on the other hand emphasize on milestone payments for any 'unproven' technology they may have taken up from the research institute. A reasonable compromise approach should be to spread the licence fee in instalments dictated by the various milestones achieved in the technology transfer process. The licensee not pursuing development of the invention towards its commercialization, has often been the cause of worry of many research insti-

tutes, including mine. If progress is not good enough, the institute should be in a position to call off the deal and find a new licensee early enough. Such intervention clauses have to be incorporated into the agreement. If the institute insists on annual licence maintenance fee, and if this quantum is increased over subsequent years, companies may be prompted to bring technologies from the back-burner and actively pursue development. Else if their interest has waned, they would request for termination of the agreement. Either way, for the institute it is better than the unpredictable wait. Successful licensing negotiations appreciate that every eventuality cannot be covered.

In the coming years technology licensing will be even more demanding. In order to be competitive, techno-enterprises may require major readjustments that call for operational flexibility and a fast response time—a trait normally not endowed to public funded research organizations. It is highly unlikely for scientists going about their usual chores to fully comprehend the implications of the law or business strategy. Their talents are best exploited in their own disciplines. The need for the hour is to nurture a band of technology transfer specialists, scientific 'dilettantes' if you may, with a hard nose for business. They should be able to sense research developments in their respective institutes that have potential for industrial applications and then successfully interface with the industry that requires this development. Most Western universities and research institutions have incorporated a technology licensing office within their organizational setup. Perhaps it is time we began to appreciate this fact and modify our approaches to suit the changing scenario.

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A non-radioactive method for detection of Tn5 Kanamycin phosphotransferase activity in cyanobacteria

The wide use of Kanamycin phosphotransferase encoding gene, also known as NPT II gene, as one of the reporter genes in transformation studies has prompted the development of a number of procedures to detect and assay Kanamycin phosphotransferase in the transformed cells¹⁻³. All procedures reported so far need the use of radioactive ATP and also adequate care to account for other phosphotransferase activities in crude cell extracts. The present report deals with a simple, non-radioactive assay for Kanamycin phosphotransferase in two genetically transformed cyanobacteria.

The cyanobacteria, *Oscillatoria* sp. MKU277 and *Westiellopsis* sp. MKU154 isolated from rice field soils were electro-transformed using pRL1063a. The plasmid contained psbA promoter from *Amaranthus hybridus* and downstream to that were five genes namely, Neomycin/Kanamycin phosphotransferase gene (Tn5), Bleomycin resistance gene, Streptomycin resistance gene and *luxA* and *luxB* of *Vibrio fischeri*. Transformants growing in BG-11 medium containing 50 µg/ml of Kanamycin were harvested at the exponential stage, washed several times with Tris (100 mM pH 8.0) containing 10 mM MgCl₂ and suspended in the same buffer. PMSF was added to a final concentration of 5 mM. Cell suspension was sonicated and the lysate was clarified by centrifuging at 10,000 g for 10 min at 4°C. The clear lysate was transferred to a fresh sterile Eppendorf tube and stored at 0°C until use. Protein content in the lysate was estimated following Bradford's procedure⁴.

Reaction mixture contained 50 µg to 100 µg crude protein, 1.0 mM ATP and 4 µg Kanamycin. Reaction was carried out at 16°C for 12 h. *E. coli* HB101 from

exponential growth phase was mixed with LB soft agar (0.7%) and spread on plates. Soon after agar solidification, sterile Whatman No. 1 filter paper discs were carefully placed on the agar surface. Equal volume of samples from various treatments were spotted on the discs. Kanamycin

with ATP alone was used as control. The plates were incubated at 37°C for 24 h and the inhibition zones were qualitatively assayed.

When the sizes of inhibition zone formed around the discs (Figure 1 a, b) were compared, there was a significant reduction in the inhibition zone around the discs containing Kanamycin treated with lysate prepared from transformed cyanobacteria, indicating inactivation of Kanamycin due to phosphorylation. Inactivation of Kanamycin depended on the amount of protein added and the time of incubation. 50 µg crude protein was required to inactivate 4 µg of Kanamycin.

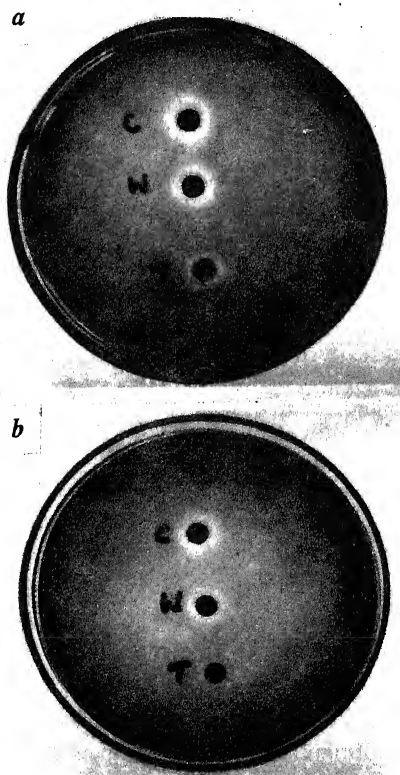


Figure 1 a, b. *E. coli* sensitivity to phosphorylated and non-phosphorylated Kanamycin. a, *Westiellopsis* sp. MKU154; b, *Oscillatoria* sp. MKU277. C – disc with untreated Kanamycin (control), W – disc with Kanamycin treated with wild type cell lysate; and T – disc with Kanamycin treated with lysates of transformed cells. pRL1063a was used for transforming the cyanobacterial strains.

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Gene identification *in silico*

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DNA sequence analysis has emerged as one of the major disciplines of theoretical biology and has become an essential tool for study in molecular biology. In this article we review various methods currently available for the analysis of genomes and large-scale DNA sequences in order to detect potential genes out of sequence information.

THE extreme diversity that exists among biological systems is essentially governed by the information content of DNA molecules that make up the hereditary elements – collectively called the genome – which are transferred from one individual to another¹. Genetic information is decoded through the ‘genetic code’, which allows specific proteins to be synthesized according to the information present in DNA.

Many features of this genetic code are by now quite well-known². DNA molecules are composed of four nucleotides, the purines adenine (A) and guanine (G) and the pyrimidines, thymine (T) and cytosine (C). The three-dimensional structure of DNA is double helical, with nucleotides on the two strands paired A–T and G–C. The actual arrangement, i.e. the sequence, and the length of the DNA differs from organism to organism and determines its complete biology. One major function of the DNA is to manufacture specific proteins, and nucleotide sequences that contain this information are called the protein-coding regions or genes. There are also ancillary regions of the DNA which regulate and control expression of the proteins at specific times and under specific conditions.

At the same time, there are vast stretches of DNA sequence, principally in higher organisms, whose function is not yet known¹. Thus, it is still not possible to predict the complete biological functions of a given organism in spite of knowing the complete sequence of its genome. Gene functions can be experimentally determined using the techniques of molecular genetics and biochemistry. Given the time taken for these experiments, though, analysis of a complete genome is still an intractable task. Moreover, for most organisms, such experimental methods and approaches are not yet feasible. Thus, the development of theoretical and computational methods^{3,4} for analysis of DNA sequences could significantly aid and accelerate understanding of functions of different regions of DNA.

Availability of entire genome sequences of organisms can help, at the very least, to establish a complete genetic map of the organisms. Further, a comparative analysis of the genomes of a given family of organisms can help determine the minimal set of genes necessary for development and organization within that family. However, the large amounts of sequence information already available and continuously pouring in (in sequence libraries or repositories such as GenBank) pose a challenge, insofar as interpretation and analysis is concerned. There are several ongoing projects to sequence the entire genome of a number of organisms. Within the next few years the intention is to have a complete genome map of organisms such as *Drosophila melanogaster* (genome length \approx 165 Mbp, consisting of \approx 15000 genes), *Escherichia coli* (4.7 Mbp, 3000 genes), *Arabidopsis thaliana* (100 Mbp, 13100 genes), nematode *Caenorhabditis elegans* (100 Mbp, 15000 genes), the puffer-fish *Fugu rubripes* (390 Mbp, 80000 genes) and the human genome (3000 Mbp, 100000 genes). Recently, the entire genomes of *Haemophilus influenzae* (1.83 Mbp, 1727 genes) and *Mycoplasma genitalium* (0.58 Mbp, 482 genes) have been sequenced^{5,6}, and the entire genome sequence of yeast *Saccharomyces cerevisiae* (12.5 Mbp, 6400 genes) is expected in a few months.

Megabasepair DNA sequencing is, by now, a well-developed technology⁵. In the standard strategy, the genome is organized into a library of overlapping fragments, randomly cloned into a number of available vectors such as cosmids, phage P1, BAC or YAC. These vectors are designed to accept large fragments of DNA so that the entire genome of an organism is represented in the library. The choice of a specific cloning vector, to a large extent, depends upon the size of a specific genome. The large fragments are further subcloned in usually M13-based plasmid vectors, to get short stretches of DNA, which are sequenced using Sanger’s dideoxy chain termination method⁷. These segments are then re-assembled to form the genome with the help of overlaps, filling of gaps, etc. To accomplish the task of assembling the sequence of a large genome in a reasonable time-span, there is need for the continual development of innovative methods, especially those leading to automation⁸, which can further accelerate the speed of sequencing. Currently, a ‘random shotgun’ method^{5,6} has been used for *H. influenzae* and *M.*

genitulum sequences where the genome was randomly cut into segments and sequenced, with alignment done entirely on computer. This approach is feasible only with short genomes or cloned DNA fragments.

The central issue, stated simply, is to identify the *functional* regions of the sequences – the genes on the genome. As mentioned before, in a complex genome, only a small part is functional, in that it is decoded into a protein with the amino acid sequence determined by the DNA sequence. Another small part performs regulatory role by determining the time and extent of decoding in the life of an organism. Protein-coding DNA, along with associated regulatory sequences, is what 'makes sense'. However, a major part of the genome is composed of highly repetitive sequences of unknown or uncertain function – which were previously termed 'junk' or 'selfish' DNA. Thus the identification of protein-coding regions on the genome becomes a major goal of DNA sequencing and sequence analysis.

DNA can be considered as a linear string of the symbols, A, T, G, and C, and it is necessary to specify the sequence along either one of the strands alone since the sequence along the complementary strand is automatically specified. Proteins are synthesized by reading a code from DNA sequence, with a triplet of nucleotides – a codon – corresponding to a given amino acid. Since 20 amino acids are the constituents of naturally occurring proteins, and there are $64 (=4^3)$ codons, the genetic code is degenerate. The elementary grammar of the genetic code also includes a rule for initiation of protein synthesis (the start codon) and a rule to signal the end (the three stop or non-sense codons) (Figure 1).

The task of gene recognition and identification poses a challenge for several reasons. One can imagine identifying genes by one of two possible routes, either from the expressed protein back to the DNA or from the DNA directly. The route from the protein back to the DNA is made difficult (and uncertain) by the fact that

the amino acid-to-codon correspondence is not unique. Since a complete knowledge of the sequences of all proteins in an organism is a distant goal, this approach is of limited utility. In any case, identifying a gene is not simply a matter of finding an open reading frame (ORF), namely a portion of DNA of length at least 100 bp which starts with a start codon and ends with a stop codon (with no other intermediate stop codons). While prokaryotic genes are often continuous ORFs, the expressed part of a gene in the majority of eukaryotes is split into several discrete segments called 'exons' which are interspersed with noncoding intermediate regions, the 'introns'. Exons may be mixed and matched in various combinations to create new genes, and sometimes one gene's exon may be another gene's intron¹. The entire gene is transcribed into an RNA molecule, from which introns are spliced out, resulting in a messenger RNA that is a continuous ORF and is translated into the corresponding polypeptide.

There are several theoretical approaches to the problem of identifying coding regions in DNA sequences. A variety of mathematical techniques have been brought into play; these include statistical analysis, stochastic modelling, dynamical systems theory and dynamical programming. Current developments in the physical sciences such as chaos theory or the study of neural networks have also been applied, with equal effectiveness. Most of these methods rely quite heavily on computational tools, and the past decade has seen the development of several computer programs that accomplish the task of predicting the coding properties of unknown sequences with varying degrees of success.

The purpose of this article is to present an exposition of the current state of the art of gene identification through computational (*in silico*) methods. In the following section, we discuss the desirable features of coding sequence finders in general, and in this context describe several of the currently employed techniques for gene identification. These methods include both prokaryotic gene detection methods which need to look for coding ORFs, as well as eukaryotic gene detectors, that must locate exons and also determine how these are to be joined in order to make a functional gene. While we have tried to describe the main methods currently employed, our list is not exhaustive; other techniques and algorithms have been reviewed recently by Fickett⁹ and Burset and Guigó¹⁰. This is followed by a discussion and summary.

Gene identification

Given a genome sequence, the task is to locate all the genes. In an ideal situation, this implies identifying all the exonic, intronic and intergenic regions, and start and stop codons pertaining to each gene.

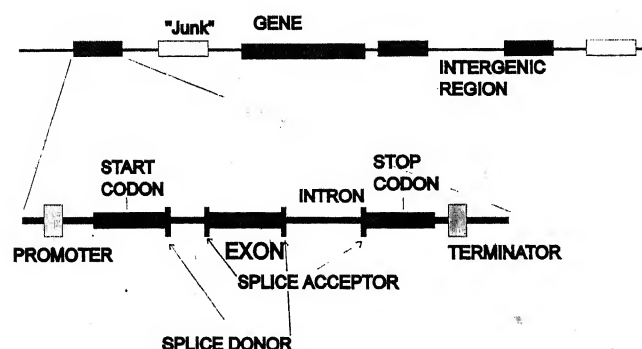


Figure 1. Schematic of the different regions in and around a gene in a genomic sequence, showing the organization of exons, introns, initiation and termination sites, intergenic spacers and promoters.

Gene-finding methods can be classified¹¹ as being *signal-based*, *content-based*, or, as is increasingly more efficient, a combination of both. Given a DNA sequence, there are six reading frames to be examined. These arise from the three possibilities for the origin of the sequence (owing to the triplet nature of the codon) and the two possibilities for the relevant strand of DNA. Signal-based techniques look for a signature in the sequences – start and stop codons, promoters, consensus splice sites, elements upstream of a gene which determine the transcription start site, polyadenylation signal at the 3'-end of the messenger RNA transcripts and similar motifs. Content methods look for codon usage biases, oligonucleotide frequencies, correlation exponents or related similar indicators.

Three measures which are used to evaluate the performance of a gene-finding technique are the sensitivity S_n , the specificity S_p , and the correlation coefficient C_c . These are defined as¹²

$$S_n = \frac{TP}{TP + FN},$$

$$S_p = \frac{TP}{TP + FP},$$

$$C_c = \frac{TP \cdot TN - FP \cdot FN}{\sqrt{(TP + FP)(FP + TN)(TP + FN)(TN + FN)}}, \quad (1)$$

where FP is the number of false positives, i.e. the number of bases *predicted* as coding, but which are subsequently, through experiments or homology, declared noncoding. Similarly, FN , the number of false negatives, is defined as the number of bases, which are experimentally confirmed as coding but are declared noncoding by the computational method. TP are the true positives, i.e. the number of bases correctly predicted as exonic, while TN are the true negatives, the bases correctly predicted as noncoding. The average of the specificity and sensitivity¹² gives some measure of the accuracy of a given method. Although the above measures are evaluated at the nucleotide level, these can similarly be defined at the exon level¹⁰.

Looking for a signal alone has its drawbacks, chief among which are the degeneracy and the lack of unequivocal definitions of signals, which can reduce the sensitivity or specificity. Looking for content alone involves the calculation of several statistical measures, the analysis of which is organism-specific, and which does not always give unambiguous results for the coding potential of a sequence.

The smallest eukaryotic genes are typically about 300 bp in length. Therefore, the existence of an open reading frame (ORF) of reasonable length suggests the *possibility* of a gene, but this alone is insufficient as

evidence of coding potential, since start and stop codons could occur randomly with some (small) probability. In addition, single base errors in sequencing cause *frame-shifts*, which may abruptly and erroneously terminate an otherwise long ORF.

'Compositional heterogeneity' is a hallmark of functional proteins. Examination of the entire protein databank (PIR database) shows that the 20 different amino acids are used in very different proportions in functional proteins (Table 1). This automatically implies that there must, similarly, be fairly stringent constraints on nucleotide usage at the level of the DNA sequence. All genomes have a bias in the base composition, possibly decided by evolution. This restricts the choice of which codons are used in designating a given amino acid. Thus coding regions have a very *unequal* usage of codons.

Constraints on the amino acid composition of functional proteins thus can lead to regularities in a coding sequence. This feature can be probed with the help of certain statistical measures, which have been used in the earliest methods to locate genes. These are discussed in the following subsection.

Oligonucleotide distribution-based methods

In these methods, 'unequal codon usage' is the fundamental measure for identifying protein coding stretches. This asymmetry in codon frequency gives rise to compositional variations. However, it has been observed that unequal usage of amino acids, without the codon preference or base composition bias, is enough to produce significant compositional variations in all the three reading frames for codons and bases. It is useful to go

Table 1. Average amino acid usage by proteins

Amino acid	Frequency (%)
Ala	7.60
Arg	5.23
Asn	4.36
Asp	5.21
Cys	1.89
Gln	4.17
Glu	6.32
Gly	7.19
His	2.28
Ile	5.29
Leu	5.81
Lys	9.17
Met	2.29
Phe	3.97
Pro	5.20
Ser	7.15
Thr	5.87
Trp	1.31
Tyr	3.21
Val	6.49

beyond the level of the trinucleotide codon and examine the distribution of other oligomers, which is also quite distinctive.

Testcode. This observation can be used to develop a method to distinguish coding regions from non-coding ones in genomes^{14,15}. The first step involves setting up a codon frequency table for coding regions, which can be achieved by using prior information coming from previously determined genes or open reading frames. Then a window is moved along the sequence, three bases at a time. For each window, the codon frequency is evaluated in each of the three reading frames, and compared to the one evaluated for a known set of genes. The frame in which the deviation is comparable to that for the test set of genes is adjudged as a coding frame, whereas if the deviation is large in all the three frames the region is identified as noncoding.

While asymmetry in codon usage has been used by Staden¹⁵ to develop a method for the identification of genes, Fickett¹⁴ exploited compositional variations observed in coding and noncoding regions. In the technique TESTCODE a total of eight parameters—four positional and four content parameters, one for each nucleotide—are used to judge whether a sequence is coding or not. The positional parameter is the ratio

$$P_{\alpha} = \text{MAX}(f_1^{\alpha}, f_2^{\alpha}, f_3^{\alpha}) / \text{MIN}(f_1^{\alpha}, f_2^{\alpha}, f_3^{\alpha}), \quad (2)$$

where f_i^{α} is the frequency of the base α at position i in the codon, and measures the extent to which a base is favoured in one codon position over another. Since it is not relevant which of the codon position favours the base, the positional parameters have fairly similar distributions in all sequences, regardless of the differences in codon usage strategy between organisms. The content parameter for a nucleotide is simply its frequency.

A standardized table, giving the coding probability of a sequence for a range of each of the eight parameters can be devised from existing sequence information available. (In Fickett's implementation of TESTCODE, this table derives from the Los Alamos Sequence Library.) Each of these parameters is used with a different weight, determined as follows: The parameter is used alone to predict coding function, from the standardized table, and the sequence deemed to be coding if the probability exceeds 1/2. The weight of the parameter is the percentage of times this guess is correct, less 50% (the random level). For a test sequence of unknown functionality, one calculates the eight parameters and obtains the probabilities p_i and weights w_i for each of them from the tables. The sum $\sum_{i=1}^8 p_i w_i$ is evaluated to get the TESTCODE indicator, and the prediction is then obtained from the standardized look-up table of the sample set.

By itself TESTCODE cannot find exact boundaries for coding sequences, but it is well adapted for combination with other techniques such as searches for ORFs, ribosome binding sites, intron boundaries. The reliability of the method when checked by taking half of the sequences in the Los Alamos Library as sample set and the other half as test set gives an error rate of prediction of around 5%.

GeneMark. Differences in oligonucleotide frequencies have been exploited in the technique GeneMark¹⁶. The Markov model is a convenient means for evaluating the probabilities of occurrence of oligonucleotides while taking into account correlations between frequencies in different positions. This model has been widely used for the study of one-dimensional strings generated in dynamical systems or those involved in language theories, to assess the correlation structure of the sequences and frequencies of words of length m in strings of length N .

A Markov process assumes that the state of a system at time t depends on its state at time $t-1$ only. This rule, when interpreted for strings of symbols, states that the probability of the symbol α_i at position i depends only on the probability of the symbol α_{i-1} at position $i-1$. For DNA sequences, this model reproduces the dinucleotide frequencies. For higher oligonucleotide frequencies, higher order Markov processes have to be invoked. For example, in a second order Markov model, the probability of symbol α_i at position i depends on the probability of the doublet $\alpha_{i-1}\alpha_{i-2}$ at positions $i-1$ and $i-2$, and this reproduces the trinucleotide frequencies in DNA.

Since correlation between nucleotides differs in coding and noncoding sequences, the corresponding Markov models are also different. The fact that the reading frame plays an important role in coding regions is accounted for by considering 'phased' or non-homogeneous Markov models. In these the probability of an oligonucleotide depends on which of the three codon positions its first nucleotide occupies. Recent studies have shown that in-phase hexamer statistics are very effective in distinguishing coding regions, since they take into account not only the codon bias but also the correlations between the various positions of neighbouring codons. Thus GeneMark uses phased fifth order Markov chains to make its predictions of coding regions.

A sliding window is used, which moves along the sequence in steps, which are a multiple of three. For each window, the algorithm calculates if the DNA fragment is modelled by the phased Markov model in one of the six frames or the ordinary Markov model. In the first-order Markov model (which can be generalized to fifth order, below), the probability that a

sequence S of length L is noncoding is given by the ordinary Markov chain formula

$$P(S|NON) = P_N^0(\alpha_1) \times P_N(\alpha_2|\alpha_1) \times \dots \times P_N(\alpha_L|\alpha_{L-1}). \quad (3)$$

Here, $P_N(\alpha_L|\alpha_{L-1})$ is the conditional probability of observing the nucleotide α_L in position L , given that α_{L-1} is observed at position $L-1$, and P_N^0 is the initial probability. P_N values are calculated on the training set of noncoding sequences.

The probability of the frame 1 in S being coding is given by the phased Markov chain

$$P(S|COD_1) = P_1^0(\alpha_1) \times P_1(\alpha_2|\alpha_1) \times P_2(\alpha_3|\alpha_2) \times P_3(\alpha_4|\alpha_3) \times P_1(\alpha_5|\alpha_4) \times \dots \times P_2(\alpha_L|\alpha_{L-1}). \quad (4)$$

Here, P_1 , P_2 and P_3 are respectively the probabilities determined for the three codon positions in frame 1 and P_1^0 is the initial probability. The values P_i and P_i^0 are defined from the training set of coding sequences. For the other frames (2–6) the probabilities $P(S|COD_m)$ are defined by similar formulae. The fifth order case is a generalization of the first order model and the probabilities are determined from Bayes formula¹⁷.

$$P(COD_m|S) = P(S|COD_m) \times P(COD_m) \sum_{m=1}^6 P(S|COD_m) \times P(COD_m) + P(S|NON) \times P(NON), \quad (5)$$

where $P(COD_m|S)$ is the *a priori* probability that an unspecified fragment S is coding and its first nucleotide is located in the codon position defined by index m . $P(NON)$ is the probability that S is noncoding and is assumed to be the same as $P(COD_m)$ and equal to 1/2.

For each sequence to be analysed GeneMark determines all possible ORFs and the average value of $P(COD_m|S)$ is computed for each ORF. If the value is greater than 0.5, the cutoff, the ORF is included in the list of predicted genes. If there is more than one reading frame in which this probability is greater than 0.5, the frame with a higher probability is chosen. When applied to the unannotated sequences of *E. coli*, the technique found many new genes¹⁷.

GeneId. The simple method described above can be made more sophisticated. For example, GeneId¹⁸, a hierarchical rule-based system for identifying probable protein coding genes, starts by identifying all possible signals, such as initiation and stop codons, donor and acceptor sites, promoters, poly-adenylation signals and assigns each a rank, according to the preferred ordering

and spacing among the various sites. Using these 'atomic sites', all possible exons are constructed and ranked by computing some of the statistically significant properties equivalent to those described above and comparing with a cutoff value obtained from a sample set. Thus each exon is sequentially filtered through the cutoff for each of the statistical measures. These exons are then classified into equivalence classes. Two exons are said to be equivalent if they occur in exactly the same gene. These classes of equivalent exons are then assembled to form the gene. A function of values assigned to each of the component exon classes is assigned to the potential gene, and this score is used to rank the gene¹⁸.

The sample sets that have been used in GeneId consist of the first, internal and terminal exons from the primate, mammalian, rodent and vertebrate groups of GenBank 64.0, excluding those with alternative splicing sites, mutants, pseudogenes, etc. These have been used to derive profiles for the prediction of the various gene-finding signals and to calculate the cut-off values for the variables used to derive the rules through which the exons are filtered. Weights are assigned to the sample set of exons to correct for the unequal representation of homologous gene families in the database. (Each family of similar exons is assigned a weight of 1.0, the weights of the sequences are assigned according to the topology of the family, and these weights are used in the derivation of the profiles.)

Given a DNA sequence, the first step involves the identification of the various atomic sites. These are confirmed by several context-based rules. For example, the first ATG does not necessarily correspond to the first AUG of the mRNA. To determine the potential start site, sequence context and distance to the cap site are used as criteria. The profile for initiation codon is derived using first expressed exons.

Similar weighted profiles are constructed for donor and acceptor sites from a set of internal exons, and respective cut-offs are established. In the case of start codons, the distance of the codon to the cap site is computed, and from the frequency distribution of this distance, a cut-off can be determined beyond which very few exons have their start. Similarly, for the stop codon, the distance to the end of transcription unit is computed for the set of last exons, and from the frequency distribution, the critical distance can be fixed. These statistics are used to establish the authenticity of a given exon.

Apart from these, some of the standard statistical measures – nucleotide frequency, positional correlations – can also be used to further filter the exons. The average correlation coefficient and sensitivity for this method, for 222 sequences from a variety of organisms – human, chicken, goat, rat, etc. – are 0.79 and 0.88, respectively.

Neural networks

Neural networks¹⁹ are numerical algorithms which allow a system to learn, recognize and classify patterns. The underlying idea for these methods derives from the nervous system of living organisms. A model neuron is a simplified version of the biological neuron, and is a two-state threshold device having outputs +1 or 0 corresponding to the firing or non-firing state of the neuron, respectively. A multiply-connected collection of model neurons forms a neural network¹⁹.

The simplest application of the collective computation of a neural network is associative memory, i.e. storage and recall of information by association with other information. This process is modelled by a neural network as follows. For a set of N two-state neurons the total number of patterns are 2^N and P of these patterns are stored in memory. If a new (or 'test') pattern is now presented to the network, it should be able to recall the stored pattern that resembles it most strongly. This is achieved by defining a dissipative dynamics on a surface wherein the stored patterns are made to correspond to local minima, and the test pattern is allowed to evolve under the dynamics so as to flow into the local minimum with the closest pattern match. The dynamical evolution of the state s_i of neuron i in the presented pattern is defined as

$$s_i(t+1) = \text{Sgn} \left(\sum_{j=1}^N w_{ij} s_j(t) - \theta_i \right), \quad (6)$$

where s_i is the state of neuron i , w_{ij} are the synaptic coupling strengths and the threshold θ_i is usually chosen to be 0, for simplicity. The choice of the weights, or coupling strengths, defines the learning rule of the network. The earliest of learning rules used is the Hebb rule, which defines the coupling strength as

$$w_{ij} = \frac{1}{N} \sum_{\mu=1}^P \sigma_i^\mu \sigma_j^\mu, \quad (7)$$

where σ_i^μ is the state of neuron i , in the stored pattern μ .

Similar to the task described above, of storing and recalling information, neural nets can be designed to recognize and classify patterns. Such nets have a fundamentally different structure, consisting of an input layer, a black-box consisting of one or several hidden layers, and an output layer. The signals received by the net through the neurons of the input layer are processed in the hidden layers, and the result is sent to the output layer. Since information flows in one direction these nets are called feed-forward layered networks or, more

simply, perceptrons. Again, in this case too the output signal depends on the input signal and the coupling strengths of the synapses,

$$S_i = f \left(\sum_k w_{ik} \sigma_k \right), \quad (8)$$

where S_i is the state of the i th neuron of the output layer and σ_k that of the k th neuron in the input layer. As before, the choice of the weights defines the learning rule. The learning is said to be supervised, if the output is known for a sample set and the weights, initially chosen randomly, are monitored by the output error. This learning is not very realistic, since it requires complete and detailed knowledge of the output, which is very uncharacteristic of the actual system they simulate. For this reason other concepts of learning have been studied, which are based on reward and penalty, for example. These come in the general class of unsupervised learning.

In supervised learning, an extensively used learning rule is the gradient learning. In this learning, the total deviation between the actual output and the desired output, and its gradient with respect to the synaptic weights, is computed. In the next iteration, the synapses are modified by a small fraction of the gradient. The disadvantage of this method is that it cannot be applied to perceptrons built with deterministic, binary-value neurons. The major advantage is that it can be generalized to multi-layered perceptrons. Multi-layered perceptrons become necessary because some very simple problems become intractable with simple perceptrons¹⁹.

A method based on the generalization of the gradient rule to multi-layered nets, is error back-propagation, when the gradient rule is applied recursively to the synapses of the output and those of the hidden layers. In applying the rule to the hidden layers, the gradient of the total output deviation with respect to the weights of the hidden layer is computed, and the weights are then modified by a small multiple of this gradient. Thus, the error at the output is propagated back to determine the weights of all the hidden layers. This is a very powerful learning rule, and has been used extensively.

GRAIL. The widely used method GRAIL employs a neural network algorithm to implement a method similar to that of GeneId. In this case, however, instead of looking for the various atomic sites, several statistical measures are evaluated and the multi-sensor neural network processes them to define a score for the coding region. As depicted in Figure 2, seven sensors are used, including Fickett's measure¹⁴, the positional base frequency to determine the coding frame, and hexamer frequencies. These are evaluated for a simple set, and

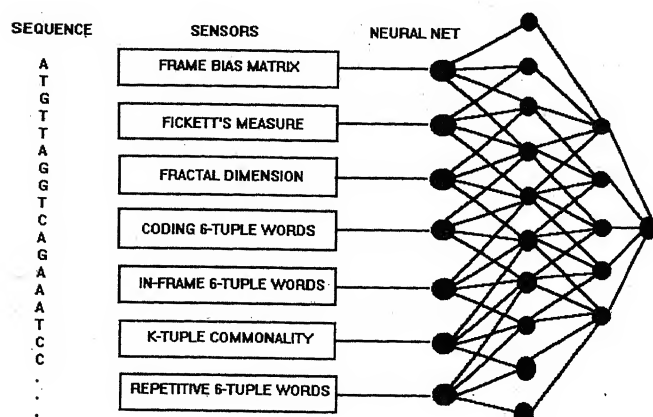


Figure 2. The neural network used by GRAIL with an input layer of seven nodes, two hidden layers of fourteen and 5 nodes respectively and an output layer of single node. (Adapted from ref. 20).

the weights of the measures are extracted. Using these weights the neural net evaluates potential coding regions.

The sample sequences originally used for the evaluation of the weights²⁰, were 18 genes from the human genome. A 99-base window, centered at each position, was used to evaluate seven sensors, scaled between 0.0 and 1.0. These values were passed through the neural net and at the end of the training, the weights of the net were extracted. The sensors used are:

1. *Frame bias matrix.* This is a 3×4 matrix containing the frequency with which a particular base occupies each of the three positions in a codon. This measure relies on the fact that while in the noncoding region or the incorrect reading frame of a coding region the distribution of positional frequencies is nearly uniform, in the coding frame there is a significant deviation from the random distribution. The standard bias matrix has been obtained from coding exons of human sequences. The correlation coefficient between the standard matrix and that of the three reading frames of each window is evaluated. The difference between the best and the worst coefficient is used as an indicator of the correct coding frame.
2. *Fickett's measures.* These are the same parameters used by Fickett in TESTCODE. The output of TESTCODE is used as the value of this sensor.
3. *Dinucleotide fractal dimension.* It is known that dinucleotide frequencies are far from random. The various dinucleotides can be grouped according to their frequencies of occurrence. It is thus possible to view a DNA sequence as a dynamic function, by examining transitions of sequential dinucleotides, i.e. asking whether the next dinucleotide belongs to the same frequency group or not. These fluctuations are characterized by a fractal dimension²¹. This dimension has a lower value in coding regions as compared to noncoding regions. Thus the

sensor value is the difference in the dimension between a reference value derived from introns and that for the test window.

4. *Coding 6-tuple word preferences.* This measure is the sum of the 6-tuple preferences, defined as the logarithmic ratio of the normalized frequency of 6-tuple words in coding and noncoding regions. It has been noticed²² that, in addition to uneven codon frequencies, there exist correlations between nucleotide positions of adjacent codons in coding exons. Hexamer, or dicodon, frequencies are a measure of both, the codon bias and correlations between nucleotides of neighbouring codons.

5. *Coding 6-tuple in-frame preferences.* This is the same as the previous measure, except that the 6-tuple frequencies are compared with the preference values of in-frame 6-tuples in coding DNA.

6. *Word commonality.* This is the logarithmic ratio of the normalized frequency to the expected random frequency of hexamers. The word commonality measure is summed over the entire window.

7. *Repetitive 6-tuple word preferences.* This measure is the same as the above, except that the comparison here is with several classes of repetitive DNA.

Sensor 1 helps to establish the correct reading frame of a coding sequence. Sensors 2–6 are various statistical measures, which are significantly different for coding and noncoding DNA. Sensor 7 is a negative indicator, since it is a statement that repetitive DNA rarely codes for protein.

These seven sensors form the nodes of the input layer to the neural network constructed in GRAIL. In addition, there are two hidden layers of 14 and 5 nodes, and an output node. For training sets, the correct output value (0 for noncoding and 1 for coding) is also provided. In the learning phase, the net compares the output value with its prediction and adjusts its weights, using the back-propagation algorithm. The net thus optimizes its performance by continuous evaluation of the output error.

When tested²⁰ on a sample set of human genes, the overall sensitivity and correlation coefficient was found to be 0.54 and 0.69 respectively. However, the performance of a later and improved version, GRAIL2 (ref. 23), on a similar data set, showed a significant improvement, with the overall correlation coefficients and sensitivity as 0.80 and 0.86 respectively.

GeneParser. GeneParser²⁴ combines the connectionist approach, adopted by GRAIL²⁰, with a recursive optimization procedure dynamic programming²⁵, to predict intron–exon structures of genes. The dynamic programming algorithm is used first to parse a sequence into basically four classes, namely introns, first, internal and last exons, subject to certain 'grammatical' constraints. In the second step, a neural network is trained to weigh

several content and site statistics, and score the intervals of interest in the sequence. Finally, the suboptimal solutions of the parsing problem are obtained and presented in a graphical format, enabling the user to identify which of the predicted splice junctions are most likely to be correct.

Like the previous technique, here also multiple lines of evidence are used to identify exons. The content statistics used in this case are in-frame hexamer frequencies, local compositional complexity, intron-exon length distributions, bulk hexamer frequencies and BLAST similarity scores²⁶. In the site statistics, splice sites and translation initiation sites were discriminated with the help of occurrence of specific nucleotides in the vicinity of the sites.

In-frame hexamers are used to determine the correct reading frame. The logarithmic ratio of the frequency of a hexamer to that of its frequency in a random sequence of the same base composition is summed over to define the hexamer score. Preferred hexamers have a positive score, while those avoided have a negative score. While in-frame hexamers are evaluated in a particular reading frame, bulk hexamer frequencies are calculated disregarding reading frames. These frequencies differ significantly between sequences of different functionality.

Local compositional complexity is quantified through the Shannon entropy²⁷ for oligonucleotides of length $L=8$, which provides a measure of redundancy. This quantity distinguishes between coding and noncoding regions by virtue of repetitive sequences which occur typically in noncoding regions. Error tolerance can be incorporated into the method by introducing random frameshift and substitution errors with predetermined error rates into the sequences of the training set.

The method was tested on 28 human sequences used by GeneId and GRAIL. The sensitivity and the correlation coefficient for this set turned out to be 0.87 and 0.78 for the latest version of GeneParser. This is a significant improvement as far as the sensitivity of the method is concerned.

Linguistic analysis

The spoken languages of the world have two remarkable features. The first is Zipf's law²⁸, which is essentially the observation that the frequency of word usage has a power-law dependence. A histogram of the total number of occurrences of each word in a text versus the rank of the word follows a power law, with an exponent $\xi \sim -1$. This is an empirical observation and is seen to hold for all languages²⁹.

The other common feature of all languages, is redundancy³⁰, i.e. words (or sentences) do not become unintelligible by the omission of some letters (or words).

This notion of redundancy can be quantified through the Shannon entropy. If $p^{(n)}(A_1, \dots, A_n)$ is the probability to find a word (A_1, \dots, A_n) in a string of length n then the Shannon entropy is defined as

$$H_n = -\sum p^{(n)}(A_1, \dots, A_n) \ln p^{(n)}(A_1, \dots, A_n). \quad (9)$$

Such analysis can also be adapted to DNA symbol sequences²⁹, if one can properly define the concept of a word. In coding regions, the 64 codons can be considered as words, but putative words in noncoding regions could have lengths greater than 3. The word of length n can be considered as a parameter that varies from three upward, and a DNA sequence is considered as an overlapping set of word n -tuples. Sequences from different categories of organisms were analysed²⁹, and the Zipf exponents were larger for noncoding sequences than for coding sequences – indeed the largest exponent obtained was for the noncoding sequences of *C. elegans*, $\xi=0.537$. This result (which is not entirely uncontroversial³¹) suggests out that noncoding regions were closer to the natural languages than the coding regions, and implies that noncoding regions may have a structured 'language'.

It is conceivable that this difference in language-like properties between coding and non-coding regions can be adapted to a method to distinguish between coding and noncoding regions, similar to the coding sequence finder (CSF) described in section 'Coding sequence finder' below. However, linguistic analysis-based methods for gene identification, on the other hand, exploit the formal structures of languages and grammars.

GenLang. Formal language theory views languages as sets of strings defined over some alphabet with concise sets of rules called grammars. Theoretically, for a given alphabet one can define an infinite number of languages. Grammars have been studied intensively with the help of computers, and have been used to describe the complex structures of strings of symbols. In the process computer programs, called parsers, have been developed, which are capable of determining whether a given string satisfies the rules of the grammar. These programs have been applied to the problem of searching for complex patterns specified by grammars, in a technique known as syntactic pattern recognition.

The information encoded in the DNA sequences uses an alphabet of four letters, with a set of rules determining the protein-coding regions. Thus the techniques of language theory can be applied to identify the syntactic patterns of the DNA language. The problem consists in defining the protein-encoding gene grammars. The core of the grammar¹² can be presented in the form of a binary tree (Figure 3). The root node is the gene, which is hierarchically built up. It is analogous to the sentence of a natural language. It consists of a start (and body)

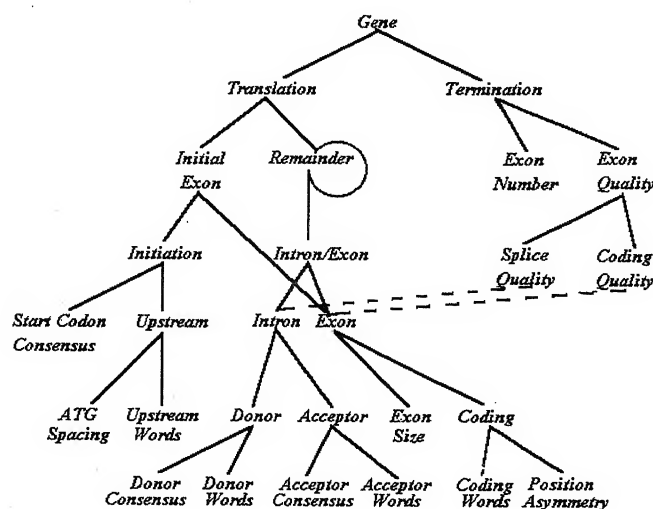


Figure 3. Graphical representation of the core grammar structure used in GenLang. (Adapted from ref. 12).

and a termination. There are various rules which define the start (first exon) and the body (internal exons and introns), and the termination (last exon). At the bottom of the tree are what are termed as 'leaf' rules, variously called sensors (neural network technique) or 'prelexical' in the linguistic context. These are derived directly from the sequence. There are 13 leaf rules used in GenLang, including rules for determining a true initiation of translation, such as consensus for the start codon, its distance from the ATG preceding it upstream, etc.

Each leaf rule is assigned a 'cost', or a threshold error, which it can accept. The cost of consensus sequence (signal), for example, is defined as the sum of the negative logarithm of individual base position frequencies, normalized so that the most likely base in each position contributes zero cost. Costs are propagated up the parse tree and summed at each node, which is connected to two leaves or other nodes, so that each subtree has its own threshold cost. The cost at node N is defined in terms of the costs of the two lower-level rules—left (L) and right (R) child—as follows

$$\text{Cost}_N = (1 - \mu) \text{Cost}_L + \mu \text{Cost}_R, \quad (10)$$

where μ is a mixing parameter, with values ranging between 0 and 1. As for the neural networks, values of μ are determined from sample sequences. In addition, each node is associated to a pair of threshold costs, θ_L and θ_R . If the cost accumulated from a subtree at a node exceeds the threshold cost, that path is discarded and the grammar may backtrack or retry the node at the next iteration. Even if a gene is developed successfully, the grammar can be made to backtrack, in an attempt to minimize the overall cost.

The training set currently used¹² consists of sequences from organisms including man, mouse, *Drosophila* and dicot plants clustered into groups of similar genes, as in GeneId, to take into account the over-representation of some classes of genes (e.g. globins) in the databank. The performance of the technique was assessed, using various measures, including the standard sensitivity and correlation coefficients. For a test set (also from the same organisms) these values were 0.83 and 0.77 respectively. The other measures were more stringent, such as the fraction of genes correctly predicted completely and the fraction of correctly predicted exons. Typical values for these were 0.1 and 0.5 respectively.

Correlation methods

One feature of all methods described above is that they are *context dependent*, i.e. in each case a sample set is required, and parameters obtained from this sample set are then used to determine the potential coding properties of a test sequence. Two approaches that do not require any particular prior information to determine the coding potential of a DNA sequence, which are based on the correlation properties, have recently been developed.

In recent years there has been a flurry of activity, primarily in the physics literature, on the study of long and short-ranged correlations in long genomic sequences. A widely used tool to study the short and long range correlation structure of symbolic strings is the discrete Fourier transform. For a symbolic sequence $\{S_j(\alpha)\}$, $j = 1, \dots, N$, of symbols $\{\alpha\}$ this can be defined as

$$S(f) = \sum_{\alpha} S_{\alpha}(f) = \sum_{\alpha} \frac{1}{N^2} \left| \sum_{j=1}^N S_{j\alpha} e^{2\pi i j f} \right|^2 \quad (11)$$

where

$$S_{j\alpha} = 1 \quad \text{if } S_j = \alpha \\ = 0 \quad \text{if } S_j \neq \alpha.$$

The analysis of a large set of coding and noncoding sequences has revealed^{32,33} that the Fourier transform of coding sequences has a distinct peak at frequency $f = 1/3$, as in Figure 4a, while this peak is absent from noncoding sequences as in Figure 4b, independent of organism and base composition. On the other hand, long-ranged correlations show up as a $1/f$ fall-off in the frequency, and this can be seen either in the simple power spectrum defined above^{34,35}, or in more involved analyses such as the wavelet transform³⁶.

Another method to detect long-range correlations proceeds through the construction of a so-called DNA walk³⁷, which is defined as follows. Starting from the origin of a square lattice, one considers a directed

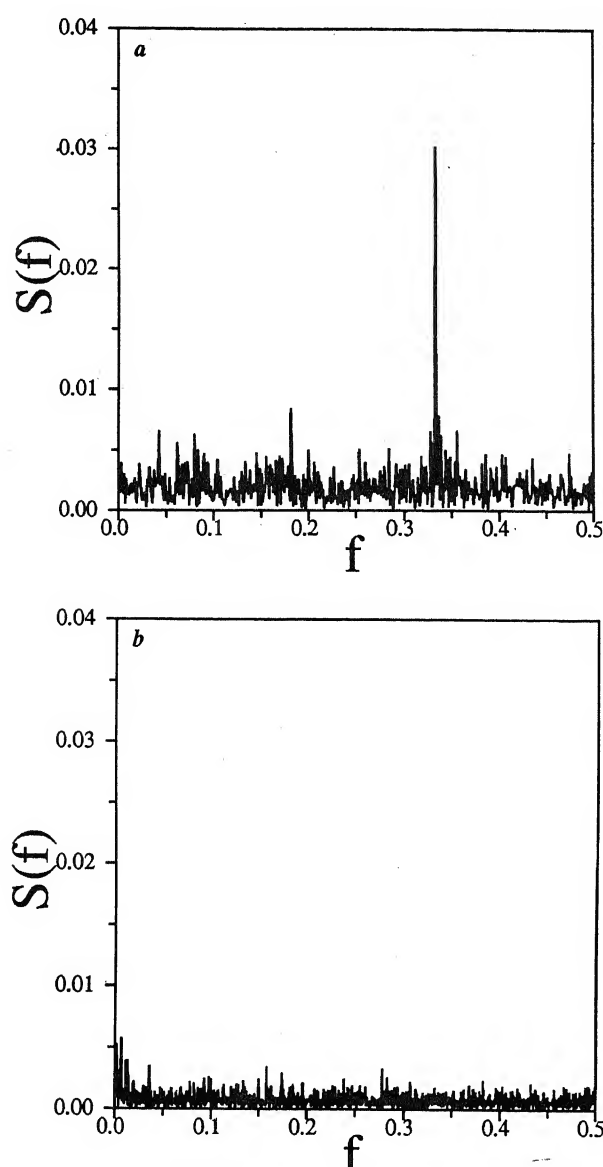


Figure 4. The Fourier transform of a coding (a) and a noncoding (b) sequence from chromosome III of *S. cerevisiae*.

random walker who takes steps up or right according to the nature of the DNA sequence under analysis. For a pyrimidine at site i , the walker takes a step upward, $u(i) = +1$, and for a purine, a step rightward $u(i) = -1$. The net displacement $y(n)$ after n steps scales as $y(n) \sim n^\alpha$. For an uncorrelated or short-range correlated walk, the theory of random walks gives the well-known result that $\alpha = 0.5$, and thus deviations from this scaling behaviour are indicative of long-ranged correlations in the data.

One difficulty of applying this approach directly to DNA sequences is that, DNA has a patchy structure, i.e. the average base composition varies from region to region, and this can give rise to features similar to those observed in long-range correlations. To overcome

this difficulty, a technique termed Detrended Fluctuation Analysis (DFA)³⁸, which involves dividing the region of interest of length N , into N/l non-overlapping windows of length l is adopted. For each window, the ordinate of the least squares fit for the net displacement of the DNA walk is defined as the local trend, and the detrended walk is then defined as the difference between the original walk and the local trend. The variance about the local trend in each window is calculated and averaged over to give a quantity $F_d(l)$. It was shown that $F_d(l) \sim l^\alpha$, where $\alpha = 1/2$ for patchy, but otherwise uncorrelated or short-range correlated sequences, while for long-range correlations $\alpha > 1/2$.

Results of studies of several DNA sequences, separately analysed for coding and noncoding regions, have established the result that coding regions have short-range correlations, while noncoding and intronic regions have long-range correlations. Although this result is somewhat controversial³⁹, there is enough evidence to suggest that the scaling exponents do indeed behave differently in the two cases⁴⁰.

The above empirical observations can be adapted as the basis of techniques to then predict coding potential, either by looking for the presence of short-range correlations, in the Fourier method GeneScan³³ outlined below, or by looking for the absence of long-range correlations, as in the coding sequence finder (CSF) algorithm⁴¹.

Another technique developed⁴² to study the correlation structure of DNA sequence uses the ideas of the so-called chaos game. The chaos game representation (CGR) of a DNA sequence can be constructed as follows. The four corners of a square are labelled by the four nucleotides in DNA, A, T, G, C respectively. Starting from the origin (the centre of the square), a point is plotted midway to the corner corresponding to the first nucleotide of the sequence. Subsequent points are the successive midpoints between the previous point and the corner corresponding to the current nucleotide, as the sequence is read through. What emerges is a pictorial representation of the DNA sequence. The density of points in the different part of the square indicates the various correlations between nucleotides. For a random sequence, for example, the square fills up uniformly. This technique has so far been used only to classify⁴³ different groups of genes. The difficulty of using the technique to recognize coding regions is that a CGR pattern becomes distinct only for fairly long sequences (> 1000 bp).

GeneScan. In earlier work³³, we have used the existence of the $1/3$ periodicity in coding regions to develop the technique GeneScan which detects the coding potential in genomic regions as follows. A window of length M , is moved along a sequence of length N , and the *local* peak-to-noise ratio at $f = 1/3$, defined as

$P_M(j) = P(1/3)/\bar{P}$, is measured. Here $P(1/3)$ is the peak height at $f=1/3$, \bar{P} is the average peak height of the spectrum and j is the position of the centre of the window. Study of a very large number of previously identified genes and noncoding sequences has shown that the peak-to-noise ratio of the spectral feature at $f=1/3$ exceeds 4 in almost every coding sequence, while for a noncoding sequence, this ratio is less than 4 (and usually less than 3). This empirical observation can be used to set a threshold, which, if the local peak to noise, $P_M(j)$ exceeds, then the window is deemed to overlap with a coding region. This simple procedure thereby gives the approximate location of coding exons. Figure 5 shows a representative result, for bases 15000–25000 of *S. cerevisiae* chromosome III. The size of the window to be used depends on whether or not we expect short (<200 bp) exonic regions. Once the approximate position of the coding region is located, the sequences are further scanned to find, in any of the six reading frames, the exact location of the initiation and the stop codon, or the location of possible consensus splice sites. For prokaryotic genes, our procedure works extremely well, and a typical result, from the analysis of the *H. influenzae* genome is given in Table 2. We have similarly studied a host of organism ranging from *S. cerevisiae* (9 of the 16 chromosomes), *E. histolytica*, *A. vinelandii*, *A. californica*, *C. elegans*, *M. genitalium*

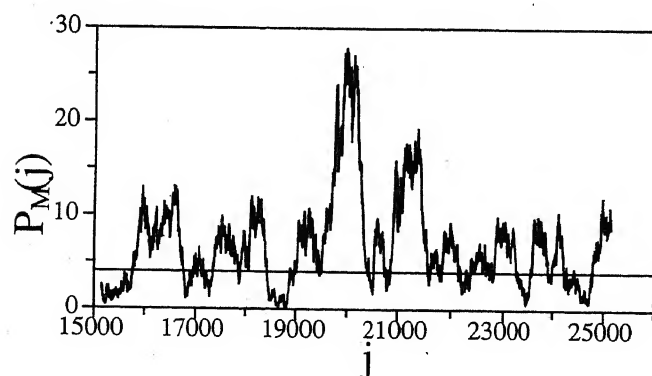


Figure 5. The results of window analysis using GeneScan on a stretch of the chromosome III of yeast. The regions where the graph exceeds the base (here taken as 4) indicate probable coding regions³³.

Table 2. Summary of results from GeneScan for the complete genome of *H. influenzae* (the quoted specificity and sensitivity are at the gene level)

ORFs reported ⁴⁵	1727
ORFs detected	1499
False positives	0
Specificity	1.0
Sensitivity	0.87
Genes reported ⁴⁶	933
Genes detected	867
Sensitivity	0.93

and several human genome sequences as well. The overall quality of the results is similar to that in Table 2.

For eukaryotic sequences, where exons can be very short, the scanning windows need to be adjusted to an optimal length. Furthermore, other techniques for locating splice sites to determine the exact location of the introns and exons also need to be used. As a consequence, the technique does not have the same level of sensitivity or specificity at the nucleotide level, for genes composed of several exonic regions³³, although the overall quality of results is very similar to that afforded by other techniques¹⁰.

Coding sequence finder. The observation of the difference in correlation exponents between coding and noncoding regions can also be implemented to scan a genome to determine the potential coding regions⁴¹. Here also, a window of length W is moved along the genomic sequence. For each window, a double logarithmic plot of $F_d(l)$ vs l is constructed. The exponent α is obtained as the (least squares) slope of this graph, and this value of α is plotted against the position of the window (defined as the centre of the window). For noncoding regions $\alpha > 0.5$, while for coding regions $\alpha \sim 0.5$. Thus a dip in the plot indicates a probable coding region and one can essentially read the coding regions off the graph; see Figure 6.

The major drawback of this method is the large size of the window required to observe long-range correlations, which sets a limit on the smallest size of coding region detectable (usually about 1000 bp). Furthermore, the boundaries of the coding stretches are only very

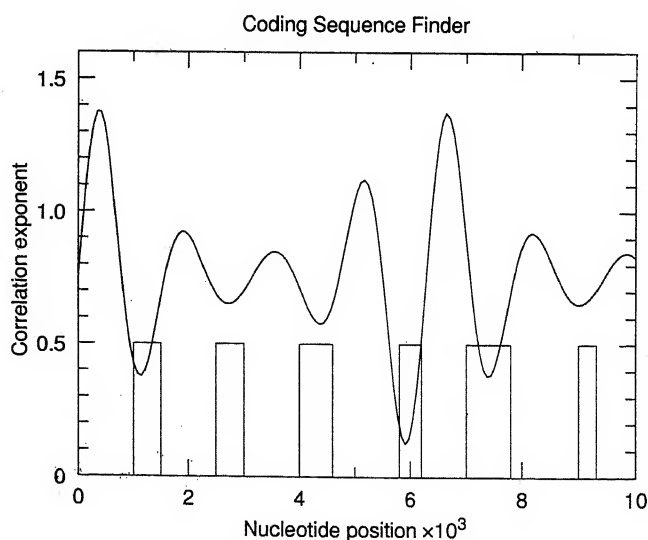


Figure 6. Typical results from the coding sequence-finder algorithm. A detrended DNA walk is constructed from a DNA sequence and the correlation exponent is determined for a stretch of about 1000 bp. The dip in the value of the scaling exponent indicates potential coding regions. (Adapted from ref. 41).

approximately determined by this method, although this can be improved by combining this with other techniques.

Discussion and summary

Rapid assessment of sequence information is possible only through computational techniques which are now being developed and refined. The different methods available have been briefly described and reviewed in the previous section, and it is clear that each algorithm or technique has something to offer.

Experience-based methods – for example those that use neural networks^{20,23,24} or prior information from Markov chain analysis^{16,18}, are currently the most widely used. These offer the advantages of pattern recognition and pattern selection. Methods that do not rely on prior information, such as those based on correlation properties^{33,41}, offer, in principle, the possibility of wider applicability. It is also likely that no single technique is complete in itself. A recent review by Burset and Guigó¹⁰ has benchmarked a variety of gene structure prediction programs against a large database, and finds that on average, the predictive accuracy of most methods ranges between 60 and 70%, for eukaryotic genes. Some techniques that are presently being developed further look for homology between the derived protein sequence and existing protein databases to completely identify genes; these are currently the most accurate methods available. However, it is perhaps unreasonable to expect that any one computer algorithm – or even a combination of several of them – will predict the location of protein coding regions with perfect specificity and sensitivity. Practical wisdom would dictate that one uses some or all of the methods available to decipher a given genome⁴⁴.

As the technology for DNA sequencing becomes increasingly sophisticated, there is bound to be a virtual flood of nucleotide sequences pouring in from a wide variety of organisms. The challenge is to make sense out of this sequence information, for the ultimate answers to the mystery of life may well lie in nature's deliberate choice of certain nucleotide sequences over others.

Data coming out from sequencing projects has brought home the realization that our level of genetic ignorance is much higher than we imagined. The example of yeast, *S. cerevisiae*, is telling. An organism which one thought was genetically beaten to pulp by decades of intensive research, has actually revealed only about one-third of its secrets to molecular geneticists. From new sequencing data – and as of today all 16 chromosomes of *S. cerevisiae* have been completely sequenced – we now know that this organism houses a much greater number of potential genes than hitherto suspected. Since it is difficult to experimentally identify genes on a mass scale given the level of technology today, it is absolutely necessary to weed out noncoding sequences (especially noncoding

ORFs) so that one has fewer potential genes for experimental verification. Computational methods do provide a quick assessment of possible coding regions, and this information can help workers know where to look for probable genes and make an experimental verification of the predictions.

At the heart of it is the problem that we still do not know *all* that makes a stretch of DNA evolve into a protein-coding sequence. Only as more and more DNAs are sequenced and analysed, will the statistics will get better and we may then develop a better understanding of why some parts in DNA are coding. Mathematical methods may also provide insights into the vast amounts of non-protein-coding DNA found in complex genomes. Is this DNA truly 'junk' or is there a pattern in it which we do not comprehend? The architectural details of genomes will provide clues to the inevitable question of how life originated and evolved on this planet.

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45. The ORFs that have been reported as coding in ref. 5, using the method GeneMark.
46. These are the genes that have been positively identified as coding through protein homology.

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Record of prolific and indubitable acritarchs from the Lower Paleozoic strata of the Tethyan Garhwal Himalaya and age implication

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Exceptionally well-preserved and prolific fossil acritarchs (a group of acid insoluble microfossils with uncertain affinities) have been recorded from the Shiala and Yong formations of the Tethyan Garhwal Himalaya, India. The presence of age marker forms of acritarch reveals that the Ordovician/Silurian boundary lies within the Shiala Formation and not at the contact of Shiala and the Yong formations, as was proposed by earlier workers^{1,2}.

THE Lower Paleozoic acritarchs are known from throughout the world, especially from UK, USA, Canada, Norway, Spain, Belgium, Southern Africa, Russia, China and Arabian Sahara. However, the record from India is almost negligible due to rare occurrence of marine Paleozoic sediments in the Peninsular India. Only the simple *Leiosphaerids*, sphaeromorphs, acanthomorphs

and netromorphs, are so far recorded from the marine Precambrian/Cambrian rocks of the Peninsular India (Vindhyan, Kaladgi, etc.)^{3–6} and the Extrapeninsular India (Krol belt, Lesser Himalaya, Tethys Himalaya)^{2,7–13}. In the Extrapeninsular India, the Lower Paleozoic marine sediments are well recognized in the Tethyan zone of Kashmir, Spiti and Kumaon–Garhwal Himalaya and contain a variety of invertebrate Paleozoic fossils.

The term 'Tethys' was conceived by Suess¹⁴ for a long expanse of Mesozoic seaway separating the old continental masses of the Gondwanaland in the south and Angaraland in the north. The 'Tethys Himalaya' refers to the widespread sedimentary basin to the north of the central crystalline rocks^{15,16}. The Tethyan sediments range in age from Precambrian/Cambrian to Early Tertiary¹⁷ and are rich in fossil contents. The Tethyan sediments of the Garhwal Himalaya have received

attention of geologists and palaeontologists since the initial work of Heim and Gansser¹⁸, but very little studies have been carried out on the palynological aspects so far, except that by Khanna *et al.*². They reported the occurrence of chitinozoans, melanosclerites, *Baltisphaeridium* sp. and other microfossils from the Yong Limestone formation of the Tethyan Garhwal Himalaya. Earlier, Khanna¹⁹ reported poorly preserved acritarch assemblage comprising nine genera assignable to 31 species from the Pin Dolomite sequence of Spiti valley, Himachal Pradesh.

The present acritarch assemblages (Figure 1) are recorded from the Shiala and Yong Limestone formations of Lower Paleozoic sediments exposed in and around the village Sumna (Lat. 30°40' and Long. 80°50'), which lies in the Chamoli district of Uttar Pradesh and close to the India-Tibet border (Figure 2). The altitude of area ranges approximately from 12,000 ft to 14,000 ft and the peaks are snow-covered throughout the year. The first author undertook two expeditions during August and September 1992 and 1993 to collect geological data and rock sample. The Sumna-Rimkhim mule track provides the excellent rock exposures and is the ideal section exposing a larger part of the Lower Paleozoic marine sequence. The exposed section comprises Garbyang, Shiala, Yong Limestone and Variegated formations.

Seventy-one rock samples at close intervals were systematically collected along Sumna-Rimkhim section (Figure 3). Strato-litho-petrographic column (Figure 4) has been prepared after careful computation and error correction of field observations²⁰ considering the aspects of topography, slope, traverse direction, altitude of exposed beds, lithology and petrographic characters. Thus, a total thickness of 680 m of the Lower Paleozoic marine sequence was systematically sampled along the section.

The integrated strato-litho-petrographic column shows age, name of formation (lithostratigraphic unit), thickness in meters, sample positions and names of rock types (Figure 4). Names of rock types, based on megascopic characters, have been given as limestone, sandstone, siltstone, siltyshale, etc. Subsequently, the precise and still more suitable names of rock types (lithounits), based on detailed petrographic study, have been given like mudstone (arenaceous), wackstone (bioclasts), boundstone (algal), grainstone (algal) and calcareous quartz arenite etc. as the classification of the sedimentary rocks^{21,22}.

Stratigraphy

A generalized lithostratigraphic framework of the Yong Gad section near Sumna has been given by Sinha²³ (Table 1), together with assemblage zones outlined by him.

All the 71 samples belonging to Garbyang, Shiala,

Yong and Variegated formations were subjected to standard maceration techniques for the recovery of acritarchs from its matrix. The heavy liquid analysis was done using KI, CdI₂ and ZnI. The samples of Shiala and Yong Limestone formations yielded prolific, varied and well preserved forms of fossil acritarchs belonging to subgroups sphaeromorphitae, acanthomorphitae, polygonomorphitae, herkomorphitae, diacromorphitae and netromorphitae. Rest of the samples from the Garbyang and Variegated formations have been found to be barren of acritarchs. The greenish grey, finely laminated silty-shale and the fine grained calcareous quartz arenite are the main lithounits which yielded fossil acritarchs from the Shiala Formation. Wackstone (bioclast) of the Yong Limestone Formation yielded abundant acritarchs which is greenish black.

This paper aims only to record the first prolific occurrence of acritarchs from the Indian subcontinent, in general, and from the Shiala and Yong Limestone formations of the Tethyan Garhwal Himalaya, in particular. All the slides and samples are deposited at the Department of Earth Sciences, University of Roorkee (India) for future reference.

Acritarch assemblages

The Tethyan acritarch assemblages are relatively smaller than the type species described²⁴. According to our observation, the size may be extremely variable depending on the direction of folding during burial and preservational history²⁵. A check-list of recovered acritarch species of Shiala and Yong Limestone formations is given in Table 2.

Age implication

The acritarch assemblage recovered (Figure 1) from the Early Paleozoic sequence of Tethyan Garhwal Himalaya is mainly dominated by the acanthomorphs, polygonomorphs, netromorphs, herkomorphs and diacromorphs. The occurrence of age marker forms such as *Baltisphaeridium longispinosum* var. *longispinosum*, *B. archaicum*, *B. citrinum*, *Deunffia monacantha*, *D. brevispinosa*, *D. monospinosa*, *Geron* sp. cf. *G. amabilis*, *Triangulina* sp., *Domasia trispinosa*, *Multiplicisphaeridium cladum*, *M. thusui*, *Leiofusa parvitatensis*, *L. algerensis*, *Veryhachium* sp. cf. *V. wenlockium*, *Stellichinatum celestum*, *Dactylofusa* sp. cf. *D. oblancae* and *Oppilatala? indianae* suggest Late Ordovician to Late Silurian age for the sediments exposed along the Sumna-Rimkhim section.

The sediments of the Shiala Formation yielded dominant to common occurrence of *Ordovician nudum*, *Multiplicisphaeridium ornatum*, *Veryhachium longispinosum*,

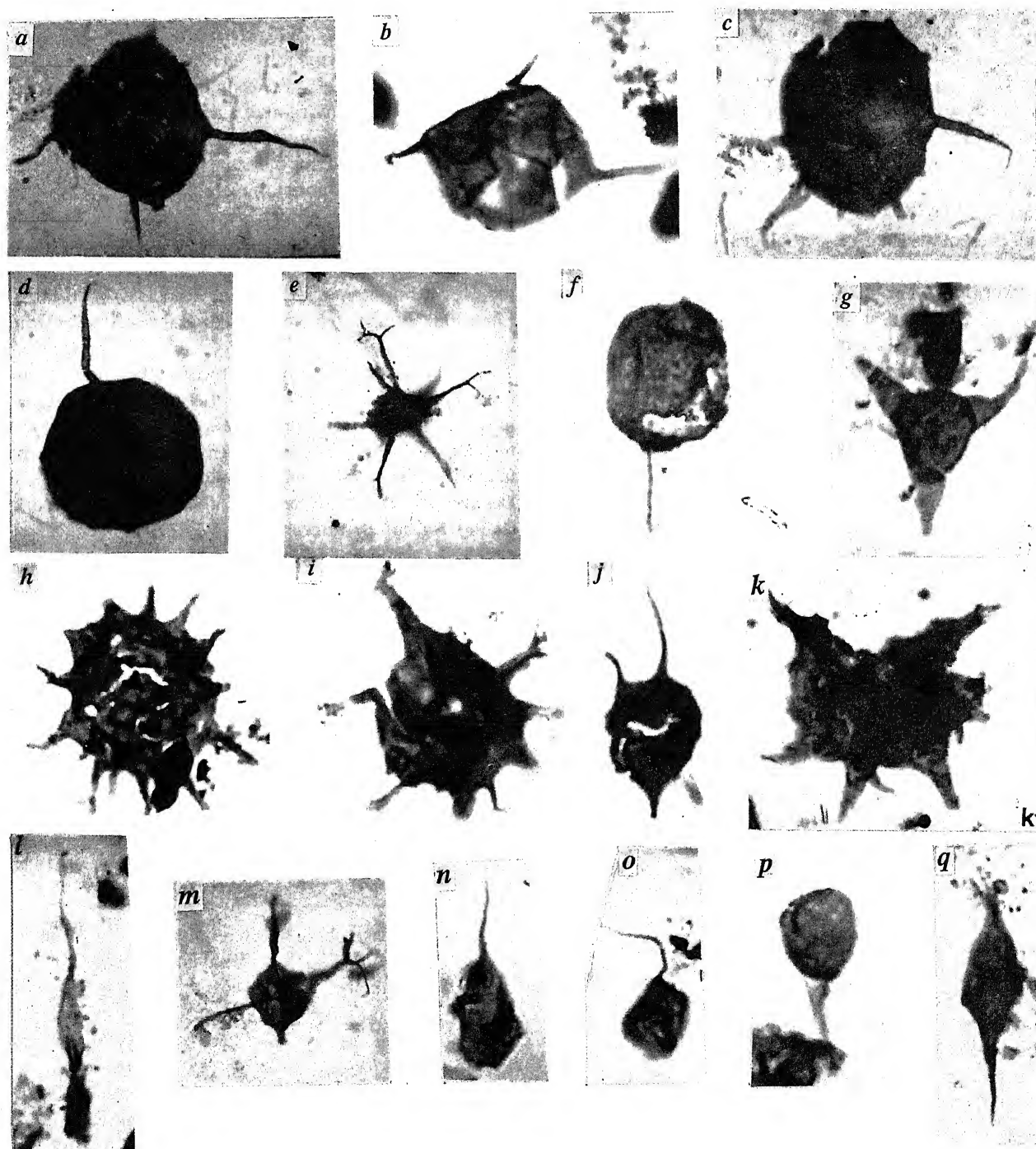


Figure 1. D = Maximum diameter of vesicle. *a*, *Baltisphaeridium archaicum*³⁴; D = 30 μ m, Slide No. R-8(2); Coord. 94.1 \times 36. *b*, *Leiofusa bernesgae*⁷⁹; Longitudinal axis = 14 μ m, Slide No. R-8(0); Coord. 104.3 \times 70.6. *c*, *Baltisphaerosum bystrentos*³⁶; D = 39 μ m, Slide No. R-8(2); Coord. 95 \times 31.3. *d*, *Deunffia monacantha*⁷⁵; D = 24 μ m, Slide No. R-8(2); Coord. 92.2 \times 64.3. *e*, *Multiplicisphaeridium variable*⁴¹; D = 15 μ m, Slide No. R-47(1); Coord. 94.6 \times 60. *f* and *p*, *Geron* sp. cf. *G. amabilis*⁷⁸; *f*, D = 6 μ m, Slide No. R-8(0); Coord. 106 \times 67.7, *p*, D = 8.5 μ m, Slide No. R-8(0); Coord. 97 \times 40. *g*, *Triangulina*⁶¹ sp.; Largest process = 7 μ m, Slide No. R-8(0); Coord. 103.3 \times 40.1. *h*, *Multiplicisphaeridium cladum*²⁸; D = 14 μ m, Slide No. R-8(0); Coord. 104.6 \times 52.3. *i*, *Multiplicisphaeridium thusui*⁴⁷; D = 14 μ m, Slide No. R-8(0); Coord. 104.1 \times 63.4. *j*, *Domasia trispinosa*⁷⁴; D = 10 μ m, Slide No. R-8(0); Coord. 97 \times 27.6. *k*, *Stellechinatum celestum*³⁶; Length of body = 12 μ m, Slide No. R-21(5); Coord. 111 \times 33.7. *l-q*, *Leiofusa parvitatis*⁴⁰; *l*, length of vesicle = 7 μ m, Slide No. R-47(0); Coord. 94.8 \times 47.8. *q*, length of vesicle = 10 μ m, Slide No. R-8(0); Coord. 106.7 \times 32.7. *m*, *Oppilatala? indianae*⁴⁹; D = 24 μ m, Slide No. R-47(2); Coord. 94 \times 30.9. *n*, *Deunffia monospinosa*⁷⁴; *n*, D = 8 μ m, Sample No. R-8(0); Coord. 102.6 \times 69.4. *o*, D = 6 μ m, Sample No. R-8(0); Coord. 99.9 \times 63.9.

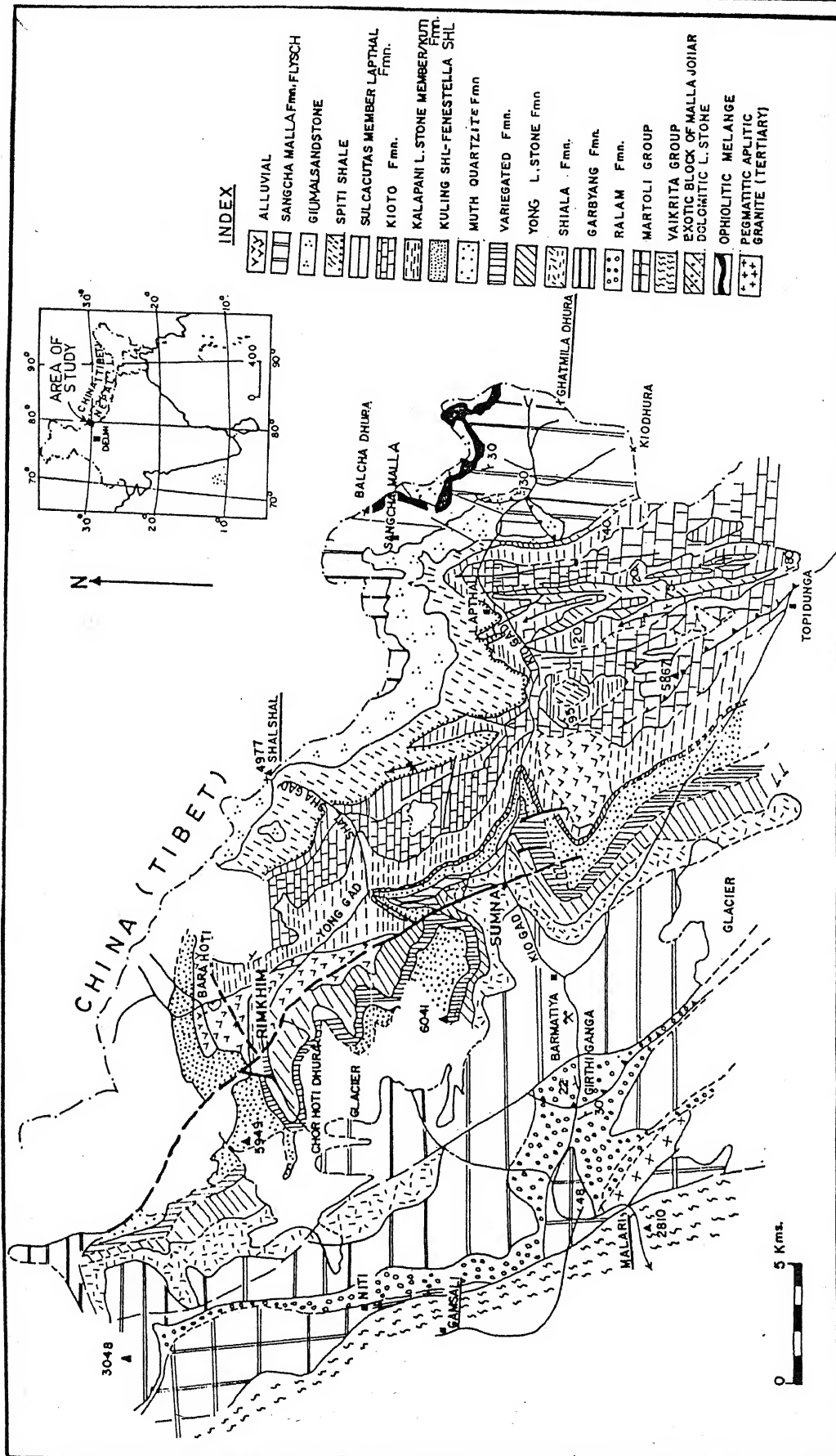


Figure 2. Geological map of the Malari-Lapthal area of Tethyan zone of the higher Garhwal Himalaya, India (A. K. Sinha, 1985).

Filisphaeridium henryi, *Neoverhachium* sp., subdominant presence of *Michrhystridium robustum*, *M. parinconspicuum*, *Acanthodiadrodium simplex*, *Solisphaeridium nanum*, *Baltisphaeridium citrinum* and *Coryphidium miladum* along with restricted occurrence of *Baltisphaeridium longispinosum* sub sp. *delicatum*, *B. archaicum*, *B. longispinosum* var. *longispinosum*, *B. bystrentos*, *Deunffia monacantha*, *D. monospinosa*, *D. brevispinosa*, *Domasia trispinosa*, *D. algerensis*, *Geron* sp. cf. *G. amabilis*, *Leiofusa parvitatis*, *Multiplicisphaeridium cladum*, *M. thusui* forms which are globally restricted to Caradocian to Wenlockian age^{28,30,33,36,37,40,44,62,75,76,80-91}.

Therefore, Caradocian to Wenlockian age is assigned to the Shiala Formation.

The Yong Limestone Formation yielded marker acritarch species, such as, *Leiofusa berneseage*, *Oppilatala?*

indianae and *Multiplicisphaeridium variabile* which have been described from Ludlovian sediments of various places^{28,34,45,80,92,93}. Therefore, Late Silurian age (Ludlovian) is inferred for the Yong Limestone Formation.

The sediments overlying the Yong Limestone Formation belong to the Variegated Formation and are observed to be devoid of acritarchs. However, the Muth Quartzite section which overlies the Variegated Formation has yielded rich assemblages of brachiopod (*Pentamerifera* sp.) of the Late Silurian age⁹⁴. The record of Late Silurian brachiopod in the top horizon of the Muth quartzite sequence suggests Ludlovian age for the Variegated Formation.

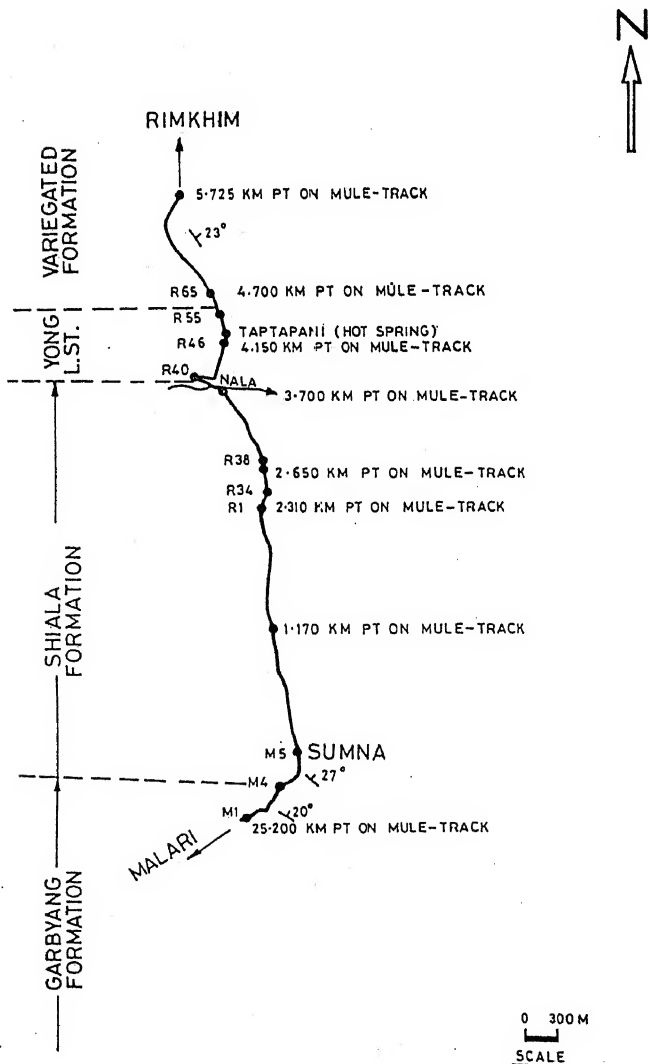


Figure 3. Sumna-Rimkhim mule-track section showing location of samples.

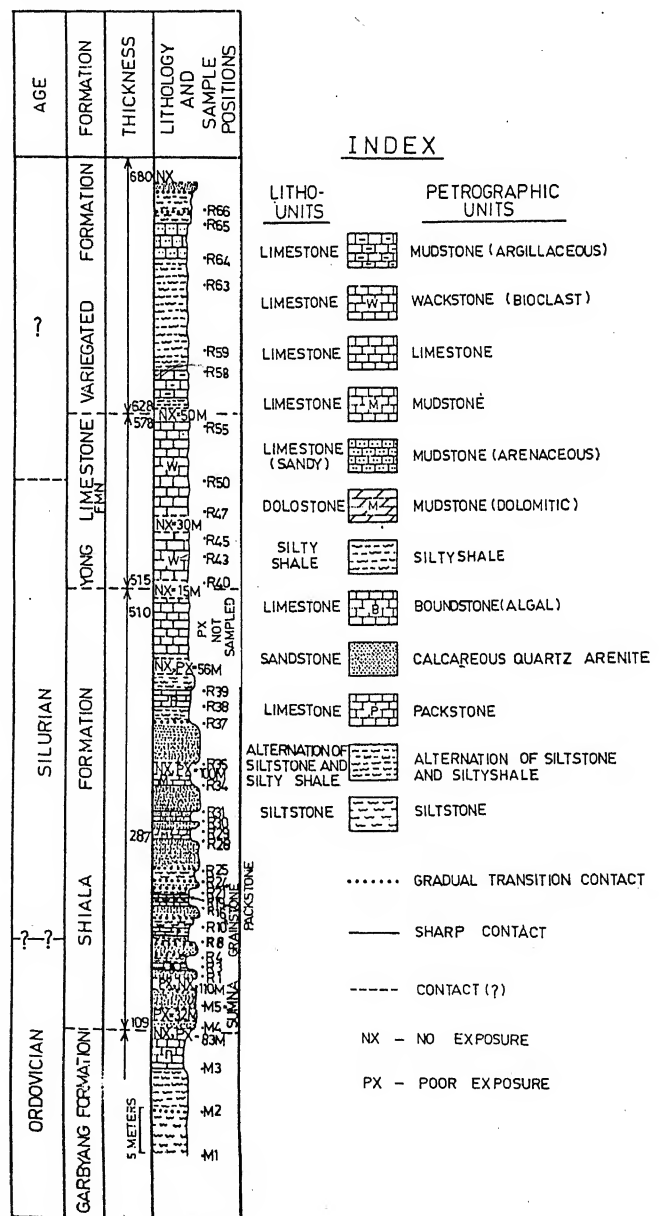


Figure 4. Integrated strato-litho-petrographic column of Sumna-Rimkhim section along the right bank of Yong Gad Valley showing sample positions.

Table 1. Generalized lithostratigraphic framework (Sinha, 1989)

Time unit	Lithounit	Broad lithology	Assemblage zones
Silurian	Variegated Formation	Purple l.st and shl. with bands of quartzite	<i>Strophonella</i> zone
	Yong Limestone	Green nodular biohermal and biostromal l.st.	<i>Calostylis</i> zone
Ordovician	Shiala Formation	Grey to pinkish s.st. quartzite, l.st.	<i>Rafinesquina</i> alternate zone
		Alternate bands of s.st. and shl.	<i>Monotrypa</i> zone
		Alternate bands of greenish shl. and biostromal l.st., splintery shl. with arenite	<i>Rafinesquina aranea</i> zone, <i>Orthis testudinaria</i> zone
	Garbyang Formation	Green needle shl. with occasional limestone	

Table 2. Check-list of recovered acritarch species from Shiala and Yong Limestone formations

Group	: Acritarcha ²⁶		
Subgroup	: Sphaeromorphitae ²⁷ , <i>Lophosphaeridium</i> ²⁸ , <i>L. parvum</i> ²⁷ ; Sample No. R-21, <i>Lophosphaeridium</i> sp.; Sample No. R-12		5. <i>Neoveveryhachium</i> ³⁸ , <i>Neoveveryhachium</i> sp. A
			6. <i>Orthosphaeridium</i> ⁵⁸ , aff. <i>Orthosphaeridium</i> sp.; Sample No. R-50
Subgroup	: Acanthomorphitae ²⁷		7. <i>Polygonium</i> ³⁸ , <i>P. delicatum</i> ³⁹ , <i>P. gracile</i> ⁵⁹ ; Sample No. R-8, 12, 47, <i>Polygonium</i> sp. A.; Sample No. R-43
	1. <i>Aremoricium</i> ³⁰		8. <i>Stellechinatum</i> ³⁶ , <i>S. celestum</i> ³⁶ , <i>Stellechinatum</i> sp. A.; Sample No. R-21
	2. <i>A. sp. cf. A. simplex</i> ³¹ , <i>Baltisphaeridium</i> ³² , <i>B. accinctum</i> ³³ , <i>B. archaicum</i> ³⁴ , <i>B. citrinum</i> ³⁵ ; Sample No. R-21, <i>B. longispinosum</i> sub sp. <i>delicatum</i> ³⁶ ; Sample No. R-5, <i>B. longispinosum</i> var. <i>longispinosum</i> ³⁷ ; Sample No. R-5, <i>Baltisphaeridium</i> sp. A		9. <i>Tetradinium</i> ⁶⁰ , <i>Tetradinium</i> sp. A.; Sample No. R-21, R-47
	3. <i>Baltisphaerosum</i> ³⁶ , <i>B. bystrentos</i> ³⁶		10. <i>Triangulina</i> ⁶¹ , <i>Triangulina</i> sp. A
	4. <i>Buedingiisphaeridium</i> ³⁸ , <i>B. tremadocum</i> ³⁹		11. <i>Veryhachium</i> ³⁸ , <i>V. calandrae</i> ⁶² , <i>V. downiei</i> ⁶³ , <i>V. lairdii</i> ⁶⁴ ; Sample No. R-21, <i>V. longispinosum</i> ⁶⁵ , <i>V. oklahomense</i> ⁴⁰ , <i>V. reductum</i> ⁶⁶ , <i>V. valiente</i> ⁶¹ , <i>V. sp. cf. V. wenlockium</i> ⁶⁶ , <i>Veryhachium</i> sp. A
	5. <i>Cheleutochroa</i> ²⁶ , <i>C. diaphorosa</i> ³⁶		12. <i>Villosacapsula</i> ⁶⁷ , <i>V. sp. cf. V. entrichos</i> ³⁸ , <i>Villosacapsula</i> sp.
	6. <i>Diexallophasis</i> ⁴⁰ , cf. <i>Diexallophasis</i> sp., Sample No. R-47	Subgroup	: Herkomorphitae ²⁷
	7. <i>Filisphaeridium</i> ³⁸ , <i>F. henryi</i> ³⁸		1. <i>Cymatiosphaera</i> ⁶⁸ , <i>C. sp. cf. C. celtica</i> ⁶⁹ , <i>Cymatiosphaera</i> sp. A
	8. <i>Helosphaeridium</i> ²⁸ , <i>H. citrinipeltatum</i> ⁴¹ ; Sample No. R-21, <i>H. sp. cf. H. clavispinulosum</i> ²⁸		2. <i>Cymatiogalea</i> ⁵⁴ , <i>Cymatiogalea</i> sp. A; Sample No. R-21
	9. <i>Micrhystridium</i> ³⁸ , <i>M. ? diornamentum</i> ³⁹ , <i>M. equispinosum</i> ³⁶ , <i>M. exiguum</i> ³⁹ , <i>M. parinconspicuum</i> ⁴² , <i>M. robustum</i> ⁴³ , <i>M. shinetonense</i> ⁴³ , <i>M. sp. cf. M. shinetonense</i> ⁴⁴ , <i>Micrhystridium</i> sp. A	Subgroup	: Diacromorphitae ²⁷
	10. <i>Multiplicisphaeridium</i> ⁴⁴ , <i>M. cladum</i> ²⁸ , <i>M. ornatum</i> ⁴⁵ ; Sample No. R-3, <i>M. osgodense</i> ⁴⁶ ; Sample No. R-47, <i>M. thusu</i> ⁴⁷ , <i>M. variabile</i> ⁴¹ , <i>Multiplicisphaeridium</i> sp. A		1. <i>Acanthodiacrodium</i> ⁷⁰ , <i>A. rotundatum</i> ⁷¹ , <i>A. simplex</i> ⁷²
	11. <i>Oppilatala</i> ⁴⁸ , <i>O. ? indianae</i> ⁴⁹ ; Sample No. R-47		2. <i>Dasydiacrodium</i> ⁷³ , <i>Dasydiacrodium</i> sp. A.; Sample No. R-47, <i>D. sp. cf. D. longicornutum</i> ⁷¹
	12. <i>Ordovicidium</i> ⁵⁰ , <i>O. nudum</i> ³³ ; Sample No. R-11, R-17	Subgroup	: Netromorphitae ²⁷
	13. <i>Peteinosphaeridium</i> ⁵¹ , <i>Peteinosphaeridium</i> sp.		1. <i>Dactylofusa</i> ⁶² , <i>D. sp. cf. D. oblancae</i> ⁶² ; Sample No. R-47.
	14. <i>Solisphaeridium</i> ⁵² , <i>S. nanum</i> ³⁶ ; Sample No. R-21, <i>Solisphaeridium</i> sp. A		2. <i>Deunffia</i> ⁶² , <i>D. brevispinosa</i> ⁷⁴ , <i>D. monacantha</i> ⁷⁵ , <i>D. monospinosa</i> ⁷⁴
	15. <i>Vulcanisphaera</i> ⁵³ , <i>V. imparilis</i> ⁵³ ; Sample No. R-11		3. <i>Domasia</i> ⁷⁶ , <i>D. algerensis</i> ⁷⁶ , <i>D. limaciforme</i> ⁶² ; Sample No. R-39, <i>D. trispinosa</i> ⁷⁴ , <i>Domasia</i> sp. A.; Sample No. R-47
	16. <i>Stelliferidium</i> ⁵⁴ , <i>S. redonense</i> ⁵⁴		4. <i>Eupoikilofusa</i> ⁶² , <i>Eupoikilofusa</i> sp. A
Subgroup	: Polygonomorphitae ²⁷		5. <i>Geron</i> ⁷⁷ , <i>G. sp. cf. G. amabilis</i> ⁷⁸ , <i>Geron</i> sp. A
	1. <i>Coryphidium</i> ⁵⁵ , <i>C. miladum</i> ⁵⁶ ; Sample No. R-17, R-12		6. <i>Leiofusa</i> ⁶² , <i>L. algerensis</i> ⁶² , <i>L. berneseae</i> ⁷⁹ , <i>L. elenae</i> ⁷⁹ ; Sample No. R-15, <i>L. parvitatensis</i> ⁴⁰ ; Sample No. R-47, <i>Leiofusa</i> sp. A.
	2. <i>Dorsennidium</i> ³⁸ , <i>D. rhomboidium</i> ³⁸ , <i>D. minutum</i> ³⁸ , <i>D. europaeum</i> ³⁸ , <i>Dorsennidium</i> sp. A		
	3. <i>Goniosphaeridium</i> ⁵¹ , <i>G. splendens</i> ³⁶ ; Sample No. R-21		
	4. <i>Impluviculus</i> ⁵⁷ , <i>I. sp. cf. I. stellum</i> ³⁹		

All the genera/species are extracted from sample No. R-8-siltyshale, unless mentioned otherwise.

Discussion

The acritarch studies of the Sumna–Rimkhim section of the Tethyan Garhwal Himalaya have provided fresh impetus to the stratigraphy of this region and revised the earlier date assigned to the Shiala Formation.

The earlier work^{2,23} suggests that the Ordovician–Silurian boundary lies within the lower part of the Yong Limestone Formation. However, the recognition of stratigraphically important acritarch species suggests that the Ordovician–Silurian boundary lies within the Shiala Formation at 2.320 km pt on the Sumna–Rimkhim mule track section (Figure 3) and at the sample position [No. R-8 (Figure 4)]. This shows that the lithostratigraphic boundary may not coincide with the biostratigraphic boundary.

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Tumour-promoting diterpene esters of the plant family Euphorbiaceae

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The plants of the family Euphorbiaceae produce highly skin inflammatory, caustic, irritant latex and seed oil. This is mainly due to the presence of various long chain esters of saturated fatty acids, attached at C-12 and C-13 positions of the diterpene phorbol and at C-3 position of ingenol moieties. These diterpene esters are strong tumour promoters in Berenblum's experiment and are well established as carcinogens. The use of croton oil for fish killing in small ponds and consumption of bitter honey, contaminated with ingenol esters, play positive role in exacerbating the overall process of carcinogenesis, among the population exposed to these tumour promoters. This observation is well sustained from epidemiological studies.

The earliest, and probably the most significant biological effect of croton oil, which is obtained from the seeds of *Croton tiglium* L., belonging to the family Euphorbiaceae and growing as leafy shrub in India, Ceylon, Philippines, Mauritius and China, was demonstrated by Berenblum in 1941. The weekly application to the interscapular skin of mice of acetone solutions of benzpyrene and this oil or benzpyrene and croton resin, caused significant augmentation of carcinogenesis, in comparison to that observed with benzpyrene solution alone¹. Although such cocarcinogenic activity had previously been shown with oleic acid², these experiments showed that croton oil was the most potent naturally occurring cocarcinogen known.

An important step towards understanding the actions of croton oil was the observation by Mottram, that benzpyrene need only be applied once in a sub-manifestational dose to prepare the skin so that subsequent multiple applications of croton oil can elicit tumours³. Based on these observations Berenblum and Shubik⁴ proposed two-stage theory of chemical carcinogenesis. Further refinement of this experiment lead it to be known as Berenblum's experiment⁵.

The initial sub-threshold dose of the carcinogen such as benzpyrene induced an irreversible initiating process, resulting in the formation of latent tumour cells. Subsequent repeated applications of croton oil or *Euphorbia* lattices, caused an epicarcinogenic or promoting process, which enabled such latent tumour cells to develop into malignant cells.

The term cocarcinogenicity is a general one which refers to all forms of augmentation of tumour induction.

The phenomenon can arise in many ways, including additive, synergistic, preparative or incomplete carcinogenic action⁶. Tumour promoters are, thus, only one type of cocarcinogen and have been classified as incomplete carcinogens since they can complete the process begun by the initiator⁷⁻⁹.

It has been demonstrated experimentally that the information, 'potential tumour cells' generated by the initiator, persist in the target tissue for a very long period, i.e. for at least 2/3 to 3/4 of the average life span of mice¹⁰. Therefore it is reasonable to assume that the growth of 'potential tumour cells' remains under the control of the surrounding cells. If, however, after initiation the target tissue is treated repeatedly with promoter, for example, croton oil or its most active constituent: 12-tetradecanoyl-phorbol-13-acetate (TPA)⁷; the growth of the potential tumour cell is prompted to yield papillomas. These papillomas become malignant even after some time due to the inherent tendency. Therefore, in addition to the stages of tumour initiation and promotion, a third stage called tumour progression, was also postulated for mouse skin¹¹⁻¹⁴ (Figure 1).

TPA (Figure 2 b) and other 12,13-diester of diterpene phorbol represented only 50% of the entire phorbol content of croton oil and the rest of the phorbol was present in the hydrophobic portion as unidentified form of phorbol-12,13,20-triester. This was demonstrated for the first time with the isolation of a new 'cryptic irritant and cocarcinogen' from the seed oil of *Croton sparciflorus*. This new substance was found to be

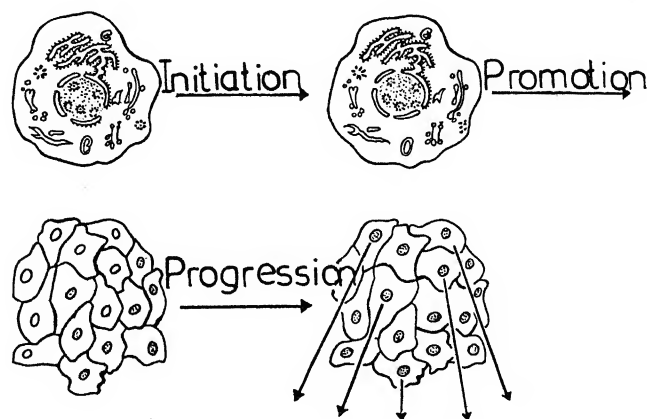
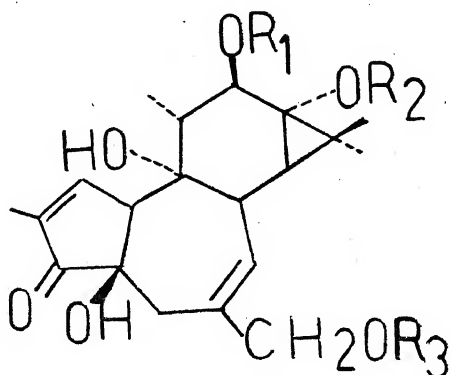


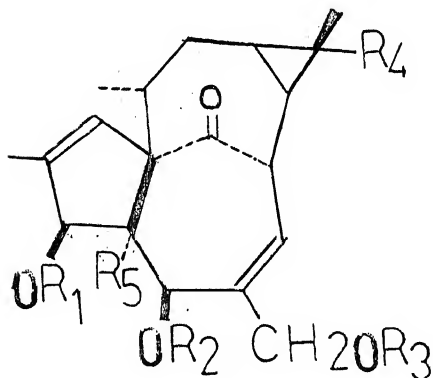
Figure 1. The three phases of tumorigenesis.

12-dodecanoyl-13-acetyl-phorbol-20-linolate¹⁵. This ester of phorbol exhibited very little irritant activity as compared to the other phorbol 12,13-diester. However, mild hydrolytic treatment of this, caused the linolinic



- a) $R_1 = R_2 = R_3 = H = \text{Phorbol}$
 b) $R_1 = \text{CO}-(\text{CH}_2)_{12}-\text{CH}_3$, $R_2 = \text{COCH}_3$, $R_3 = H = \text{TPA}$
 c) $R_1 = \text{CO}-(\text{CH}_2)_{10}-\text{CH}_3$, $R_2 = \text{COCH}_3$, $R_3 = \text{Linolate}$

Figure 2. Structure of phorbol and its esters.



- $R_1 = R_2 = R_3 = R_4 = H$, $R_5 = OH = \text{Ingenol}$
 a) $R_1 = R_2 = R_3 = \text{CH}_3$, $R_4 = H$, $R_5 = OH$
 b) $R_2 = R_3 = R_4 = H$, $R_5 = OH$, $R_1 = \text{Hexadecanoate}$
 c) $R_1 = R_2 = R_3 = H$, $R_4 = \text{CH}_2\text{OH}$, $R_5 = OH$
 d) $R_2 = R_3 = R_4 = H$, $R_5 = OH$, $R_1 = \text{Palmitate}$
 e) $R_2 = R_3 = R_4 = H$, $R_5 = OH$, $R_1 = \text{Dodecanoate}$
 f) $R_2 = R_3 = R_4 = H$, $R_5 = OH$, $R_1 = \text{Decanoate}$
 g) $R_2 = R_3 = R_4 = H$, $R_5 = OH$, $R_1 = 2\text{-methyldecanoate}$
 h) $R_1 = R_2 = R_3 = R_4 = R_5 = H$

Figure 3. Ingenol and its esters isolated from *Euphorbia* species growing in Azarbaijan province of Iran.

acid attached to position 20 of phorbol moiety (Figure 2 c) to be released selectively to yield corresponding highly active and irritant 12-dodecanoyl-phorbol-13-acetate, which is a strong tumour promoter on mouse back skin experiment. It is likely that such activation can probably take place in tissues exposed to 12,13,20-triesters¹⁵.

From the skin irritant and purgative seed oil of *Euphorbia lathyris* L. and from its highly skin inflammatory latex, esters of another diterpene, which is reminiscent of diterpene phorbol, called ingenol, have been isolated. Out of these esters ingenol-3-hexadecanoate had been found to be skin irritant and tumour promoting in Berenblum's experiment¹⁶. Further, from the skin-inflammatory and tumour promoting latex of *Euphorbia lactea*, which causes severe dermatitis and inflammation to skin, to human and animals alike, hydroxylated derivative of ingenol, called 16-hydroxy ingenol, had been isolated¹⁷ (Figure 3 b, c). In a standard experiment on the preshaved and preinitiated back skin of NMRI mouse, 0.1 μm of 7,12-dimethyl benzantracene in acetone produced squamous cell carcinoma¹⁷ after 28 weeks.

The caustic, skin irritant and tumour-promoting latex of *Euphorbia serrata* L., which grows profusely around Tabriz, capital of Azarbaijan province of Iran, and the latex of *Euphorbia seguieriana* Neck. obtained from the same region, were found to contain parent diterpene ingenol^{18,19}. However, in tumour-promoting experiments on preshaved back skin of NMRI mouse initiated with dimethyl benzantracene in acetone as usual, the latex preparation of *Euphorbia serrata* produced squamous cell papilloma within a short span of 16 weeks, due to the presence of ingenol-3-palmitate in the latex sample²⁰ (Figure 3 d), whereas the latex preparation of *Euphorbia seguieriana* Neck. produced squamous cell papilloma within 14 weeks, on the back skin of NMRI mice.

The plant of *Euphorbia esula* L. is known to cause sheep mortality and produces inflammation with the loss of hairs from the feet of the horses. The latex causes blistering with severe irritation if allowed to remain on the skin and it can lead to partial blindness if dropped into the eyes²¹. From this latex highly skin inflammatory unsaturated Δ -2,4,6,8,10-pentene-tetradecanoic acid, esterified at C-3 position of diterpene ingenol has been isolated. However this ingenol ester is nontumour promoting²² in mouse back skin experiments. From this latex another ester of ingenol, though less irritant yet tumour promoting on mice skin, was found to be ingenol-3-dodecanoate (Figure 3 e). This produced squamous cell papilloma in a short duration of 20 weeks²². *Euphorbia striatella* Boiss, which not only contained highly inflammatory latex but has various medicinal properties, was found to contain highly skin irritant, but nontumour promoting ingenol- Δ -2,4,6-decatrienoate²³. In tumour-promoting experiments on back skin of NMRI mice, the latex promoted the formation of

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squamous cell papilloma; due to the presence of ingenol-3-decanoate, within 22 weeks²³ (Figure 3f). *Euphorbia virgata*, which grows profusely in the Saidabad area of Tabriz, have been found to contain highly skin irritant latex, from which non skin irritant esters of diterpene ingenol, such as ingenol-3-phenyl acetate, and ingenol-3-benzoate were isolated. However, the irritant activity of the latex was represented by ingenol-3-tiglate and ingenol-3-(2)-phenyl decanoate. Out of these two ingenols 3-(2)-methyl decanoate (Figure 3g) was found to promote the process of tumour formation on mouse back skin²⁴. From another plant, viz. *Euphorbia megalantha* Boiss, the latex of which is moderately skin inflammatory; ingenol and a new diterpene derivative of ingenol called 4-deoxy ingenol, has been isolated²⁵. The structure of this ingenol derivative is shown in Figure 3h.

In continuation of our studies on skin irritant and tumour-promoting diterpene esters from the plants of *Euphorbiaceae* family, growing in Azarbaijan province of Iran²⁶, out of the 19 species, with curious exception of *Chrozophora tinctoria* Jass., all species were found to contain no cryptic irritant, but short chain esters of diterpene ingenol only²⁷. Long chain esters were identified as usual and for the short chain esters of ingenol, gas chromatographic techniques were found highly suitable²⁸, after these were purified by column and thick layer chromatography in the standard way^{29,30}.

It has also been shown that ingenol-3,5,20-triacetate (Figure 3a) which is totally ineffective as tumour promoter and weakly skin irritant produces in initiation promotion experiment lung adenoma in NMRI mice instead³¹. The seed oil of *Croton tiglium* L. is used by natives in India, for fish killing in small ponds, which contain mostly mud dwelling, air breathing, hardy fish *Heteropneustes fossilis*, along with other fish species³². The other plants of *Euphorbiaceae* are also employed by the tribals of Southern Rajasthan for stupefying the fish in ponds³³. These facts prompted us to take up the freshwater fish, *Heteropneustes fossilis*, as a model to study the process of liver carcinogenesis in this species.

Further, the seed oil of the plants of the family *Euphorbiaceae* growing around Faizabad was screened for its toxic effect on *H. fossilis*, and among all oils tested, the oil of *Croton tiglium* was found to be highly toxic to this fish species³⁴. Interestingly, the acetone preparations of some latex sample of the *Euphorbia* species growing in abundance around Ayodhya-Faizabad, showed strong anti-termite effects³⁵.

It has been demonstrated that administration of 0.1 μ M of 7,12-dimethyl benzantracene in tricaprylin, pro-os to this fish, only once, followed by oral administration of croton oil in tricaprylin repeatedly, triggered the formation of marked basophilia in the liver³⁶. The formation of basophilia along with other changes in the

liver were also noted when initiation has been done as recorded above and promotion was performed by pricking the liver of this fish repeatedly at fixed intervals³⁷. Various cellular changes such as hyperplasia, thickening of cell wall, loss of cytoplasm, vacuolization, nuclear displacement, and very high degree of mitosis coupled with marked basophilia, which was indicative of preneoplasia leading to hepatocellular carcinoma formation³⁸, were noted, when these fish were exposed under suitable conditions to 7,12-dimethyl benzantracene (in high doses)³⁹, 2-acetyl aminofluorene, urethane and methyl carbamate-1-naphthol⁴⁰. The Godrej hair dye, permanent black, liquid preparation, produces carcinogenic symptoms in the liver of this fish⁴¹, whereas edible dyes such as Sun Set Yellow and Brilliant blue, induce degenerative changes in the liver of *Heteropneustes fossilis*⁴². Interestingly, some synthetic strained ring compounds, having marked pharmaceutical applications, were also found to induce marked basophilia in the liver of *H. fossilis*⁴³, as did HCH (ref. 44).

It is now well established that tumour promoters make up a group of compounds largely differing from one another in chemical structure that, though by themselves non-carcinogenic, enhance the development of malignant transformation in carcinogen-initiated parenchymal and stromal cells of various organs⁴⁵. Tumour promoters also evoke a pleiotropic-hyperplastic response and several phenotypically neoplastic features in normal, i.e. non-initiated cells⁴⁶. The complex operative biological mechanism of such agents and the relation of their manifold biological effects to the actual promotion of neoplasia are the current subjects of intense investigations. Several early metabolic events, like the induction of a pro-oxidant state and of enzymatic changes at the plasmalemmal level, the stimulation of metabolism of phospholipids and of arachidonic acid, the biosynthesis of polyamines etc. seem to play a relevant role in the enhancement of the responses triggered by tumour promoters⁴⁷.

The transmembrane Ca^{2+} fluxes and events requiring the activation of Ca^{2+} binding sites, of calmodulin modulated enzymes and of ubiquitous Ca^{2+} activated phospholipid dependent protein kinase-C seemingly play a key role in the control of normal and abnormal cell proliferation and also of differentiation and functioning⁴⁸⁻⁵². It is a well-established fact that TPA and tumour promoters of marine origin such as teleocidene, lyngbyatoxin-A and aplysiatoxin induce tumour promotion through the activation of protein kinase-C. In this process the amounts of phosphoproteins increased and then expression of early response genes such as *C-fos* and *C-jun* was initiated⁵³.

Thus, the early event of tumour promotion is the increase of phosphoproteins and these act as signals for gene expression resulting ultimately in induction of clonal growth of the initiated cells^{54,55}, and are possibly

involved in the inhibition of apoptosis in tumour promoting phase⁵⁶.

Although induction of Epstein-Barr virus by these tumour promoters of phorbol and ingenol type is well established⁵⁷⁻⁵⁹, procedure for detection, but the development of basophilia in the liver of *H. fossilis*, can also be used for detecting the presence of these tumour promoters in polluted waters^{60,61} on one side and on the other it indicates the unsuitability of such fish for human consumption, as has been the case with bitter honey, collected from Saidabad area of Azarbaijan. In Saidabad area, the plants of *E. sigueriana* grow profusely and honey bees collect the nectar from the flowers. This honey tastes bitter, as it is contaminated with the tumour promoters of ingenol type⁶². The consumption of such honey appears to be the main cause for increased risk of oesophagus cancer, in hilly regions of Azarbaijan province of Iran, a fact well sustained from the epidemiological studies^{62,63}.

It has also been shown that the high incidence of oesophageal cancer on the Caribbean island of Cura-cao is mainly due to the consumption of Welensali-tea, made from *Croton flavens* leaves from which phorbol derivatives exhibiting tumour promoting activity in the oesophagus have been isolated⁶⁴. These tumour promoters contaminate indirectly milk and meat when the livestock food get mixed with the plants of Euphorbiaceae⁶⁵.

Likewise, environmental carcinogens other than those emanating from the Euphorbiaceae family, include algal microcystins, which are known to contaminate the ditch water and enhance the chances of liver cancer in certain parts of People's Republic of China^{66,67}, indicating the role these tumour promoters play in exacerbating the total carcinogenic load of the environment.

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RESEARCH ARTICLES

Submarine terrace limestones from the continental slope off Saurashtra–Bombay: Evidence of Late Quaternary neotectonic activity

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Bathymetric and shallow seismic data from the continental slope off Saurashtra–Bombay indicate wide submarine terraces at 130, 145 and 170 m and reefal structures at 320–360 m water depths. 10 cm thick slabs of limestones are recovered from the 130 m depth terrace. Some of these limestones consist of thin micrite layer on the top and a sandy layer below and others are similar to calcarenites. They contain > 95% aragonite and minor high- and low-magnesium calcite. Acicular aragonite cements occur as isopachous crusts. Dissolution and clotting of aragonite needles and drusy calcite in the interstices indicate cementation of the limestones at intertidal conditions. The age of the limestones is 11,900 years BP. These imply that the 130 m depth terrace was at intertidal depths at about 12,000 years BP. The eustatic sea-level, however, was at –90 m at 12,000 years BP. This disparity suggests neotectonic activity and subsidence by about 40 m on the Saurashtra–Bombay region some time after 12,000 years BP.

SUBMARINE terraces are important geomorphic features on the continental margins and may record former sea-levels¹. Submarine terraces have been reported^{2–4} on the western continental shelf of India between 35 and 115 m and also between 115 and 170 m water depths on the continental slope off Saurashtra–Bombay⁵.

Saurashtra was tectonically unstable during the Pleistocene^{6–9}. The eustatic sea-level¹⁰ low during the Last Glacial Maxima (LGM) was only –120 m. The deeper terraces may therefore have implications to neotectonism and Quaternary sea-level changes. We report here the investigations on the limestones from a 130 m depth submarine terrace off Saurashtra–Bombay and provide evidence of Late Quaternary neotectonic activity, hitherto unknown from the western offshore.

Materials and methods

During the cruise 150 of the *R. V. Gageshani*, bathymetric and shallow seismic data were collected from the continental shelf and slope off Saurashtra–Bombay. Sediment and limestone samples were collected with a Peterson grab (Figure 1). Mineralogy of the representative samples was determined by X-ray diffraction. Freshly broken surfaces of the limestone fragments were examined under a scanning electron microscope (JEOL T20). Radiochemistry of the limestones was carried out at the Hydrogeology and Isotope Geochemistry Laboratory, University of Paris, Orsay. Polished and thin sections of the limestones were studied under petrographic microscope.

Results

Geomorphology

The width of the continental shelf off Saurashtra ranges from 110 to 200 km. However, in the southern part, it is about 160 km wide and the shelf break occurs at 100 m water depth. Well developed 1.5 to 2.0 km wide terraces occur at 130, 145 and 170 m water depth on the continental slope (Figure 2 a and b). Several seismic profiles across the shelf and slope off Saurashtra-Bombay indicate that these terraces are major topographic features on the upper continental slope. A 15–20 m thick acoustically transparent clayey sand layer occurs landward of

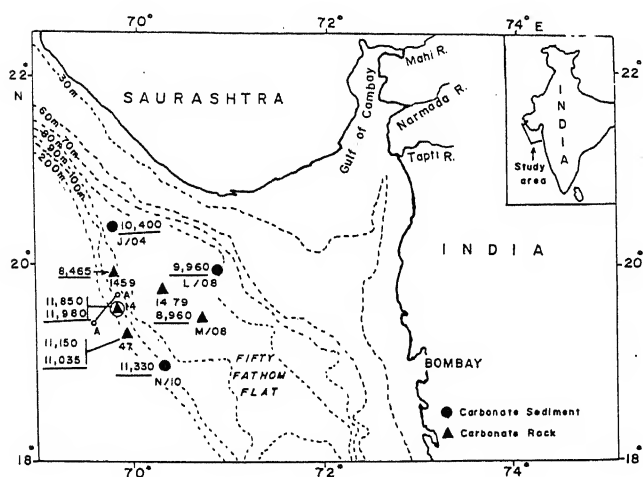


Figure 1. Sample location map (Saurashtra-Bombay). Sample collected at station 14 has been studied in this paper. Other radiocarbon dates of the samples (available in this area) are also shown and underlined. Line A-A' indicates the position of the shallow seismic (3.5 kHz ORE subbottom) profile shown in Figure 2 a.

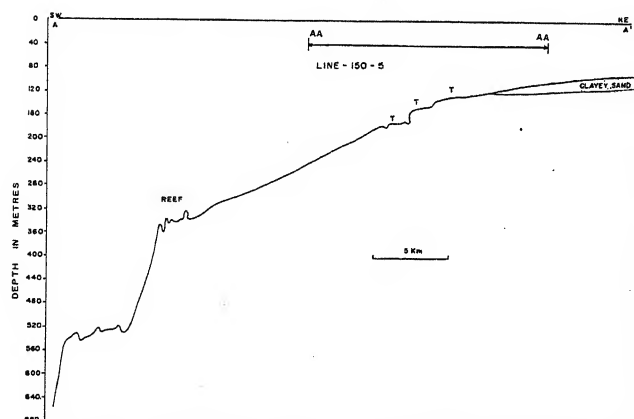


Figure 2 a. Seismic profile at the shelf break-upper continental slope showing the terraces (T) with terrigenous clayey sand zone on landward and reefal structures on seaward (see Figure 1 for location A-A').

the terraces and pinches out at 120 m depth (Figure 2 a, b). Terraces show strong acoustic reflections in seismic profiles, may be due to the exposure of relict carbonate sands and limestones. Further seaward 2 km wide and 25 m high massive reef was found at depths between 320 and 360 m (Figure 2 a).

Mineralogy and radiochemistry of the limestones

The limestones recovered from 130 m depth terrace are 10 cm thick slabs; their lower surfaces are flat and light brown and upper surfaces contain 1 cm diameter macroborings, serpulid encrustations (Figure 3 a) and ferruginized coatings. Associated sediments consist of 1–5 cm size pelecypod shells, limestone fragments and a few coral pieces. The limestones contain > 95% aragonite and minor high and low-magnesium calcite. The radiocarbon ages of the micrite-dominated and pellet-rich limestones are $11,980 \pm 185$ BP and $11,850 \pm 210$ BP and $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of these two limestones are +4.03‰ and +3.75‰ and +1.66‰ and +1.64‰, respectively.

Petrology of the limestones

Polished sections of some limestones show large borings filled with fine-grained carbonate mud (Figure 3 b) and in others dense and loosely cemented areas leading to different textures (Figure 3 c). Some other limestones contain abundant shell fragments in their upper portions. The limestones can be divided into micrite-dominated and pellet-rich types.

Micrite-dominated limestones consist of thin micrite-dominated layer on the top and sandy layer below. The micrite layer is laterally discontinuous and consists of trapped carbonate debris, partly to completely micritized algae (Figure 4 a, b) and a few pellets. Pellets are abundant in the sandy layer and cement is again micrite. Patches of micrite (Figure 4 c) consisting of oolite aggregates, cemented intraclasts and terrigenous particles are present. Pellet-rich limestones are calcarenites. Pellets (Figure 4 d) are the most abundant constituents followed by oolites, shell fragments, algal fragments with minor echinoids and benthic foraminifers. Many are mature oolites with several well developed concentric laminae. The nuclei are mainly pellets, sometimes skeletal fragments. Some oolites are bored and infected by microbial activity and their concentric laminae are riddled with a discontinuous zone of sparry calcite (Figure 4 e).

Boring cavities often impart a porous fabric to the rock (Figure 4 d). Some of the cavities are filled with terrigenous clay. Carbonate intraclasts and/or quartz and clay aggregates (Figure 4 e) commonly occur in the intergranular spaces. Acicular aragonite forms dense isopachous crusts around grains (Figure 4 f). Aragonite

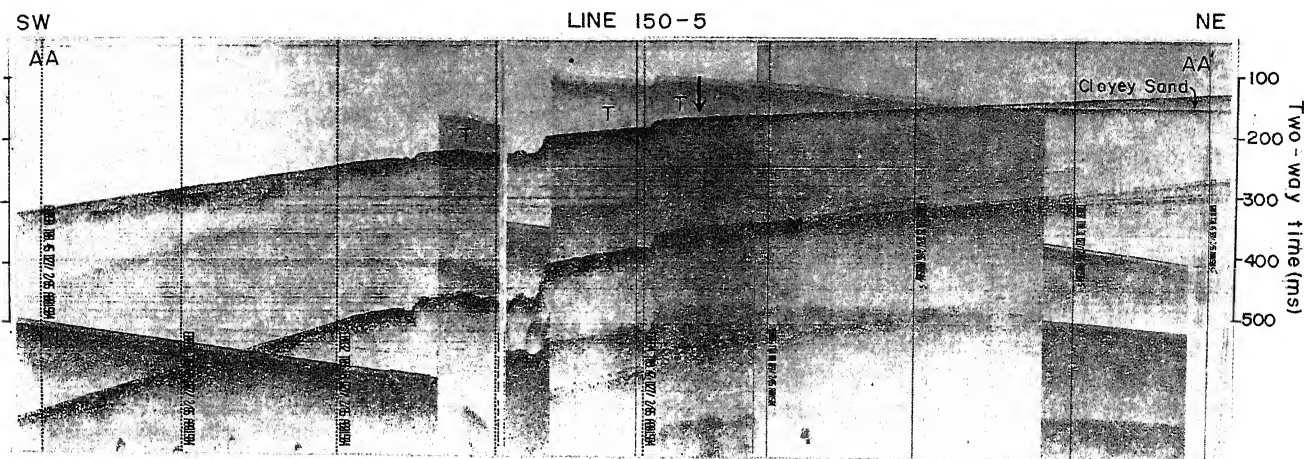


Figure 2b. Seismic profile showing submarine terraces (T) and pinching out of clayey sand zone on landward of the terrace from the upper continental slope off Saurashtra-Bombay. Arrow indicates approximate location of the limestone sample on the terrace which is 1.5 km south of the profile. M-multiple, (see Figure 2a for location AA-AA').

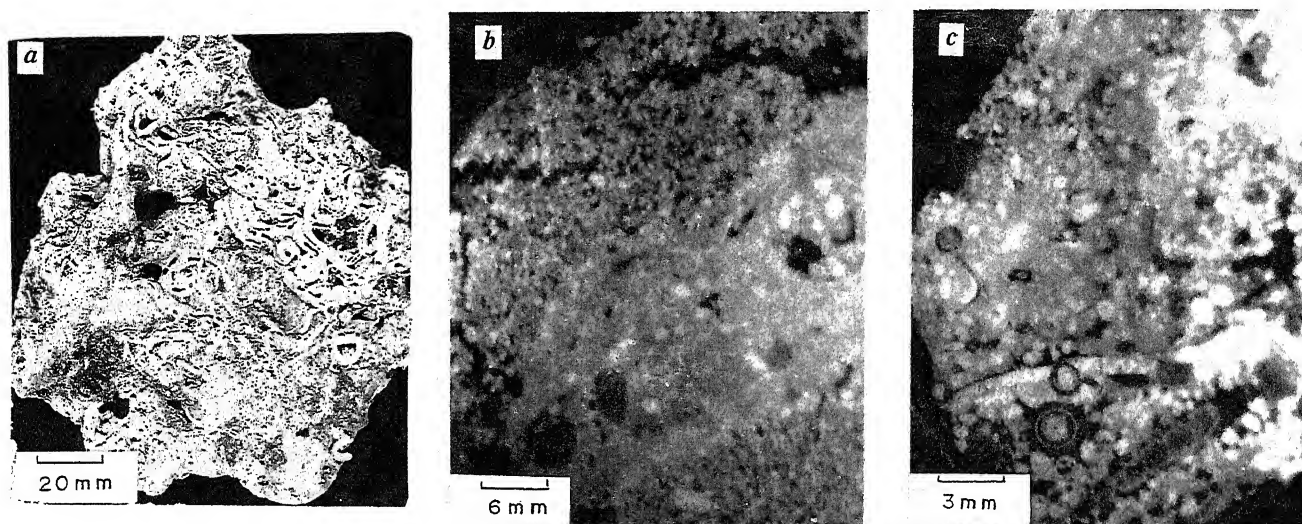


Figure 3a. Hand specimen of a limestone showing boring cavities and thickly encrusted serpulid worm tubes; (b, c) polished sections: b, the boring cavity filled with aragonite muds; c, dense and loosely cemented patches in the limestones, leading to different textures.

cements are locally dissolved and replaced by the development of calcite spar and their growth was apparently terminated before the introduction of clay in the pores (Figure 4f). Peloidal micrite cements are rare.

SEM studies on limestone fragments indicate that micrite is an aggregate of 1–2 μm globular carbonate particles (Figure 5a). Needle aragonite fringe cements (Figure 5b) are abundant. Matting of aragonite needles leading to drusy calcite (Figure 5c), dissolution of aragonite needles and the formation of calcite aggregates (Figure 5d), peloids consisting of mixture of micrite and aragonite needles and spherical aggregates of micrite are present. Globular aggregates of calcite occur within the boring cavities (Figure 5e) and sometimes attached to microbial filaments within the interstices (Figure 5f).

Discussion

Environmental conditions during sediment accumulation and cementation

Abundant shell fragments and pellet-rich sediments with some oolites in some limestones indicate high energy conditions prevailed during sediment accumulation. Colonization of both algae and lichens can produce laminated crusts on the upper surfaces of the rocks. Lichen produced crusts are, however, amorphous¹¹. The laminated micrite crusts with trapped detritus and algal material (Figure 4a, b) may therefore suggest algal colonization by trapping and binding activity and their subsequent calcification to micrite. Algally formed micrite laminations in

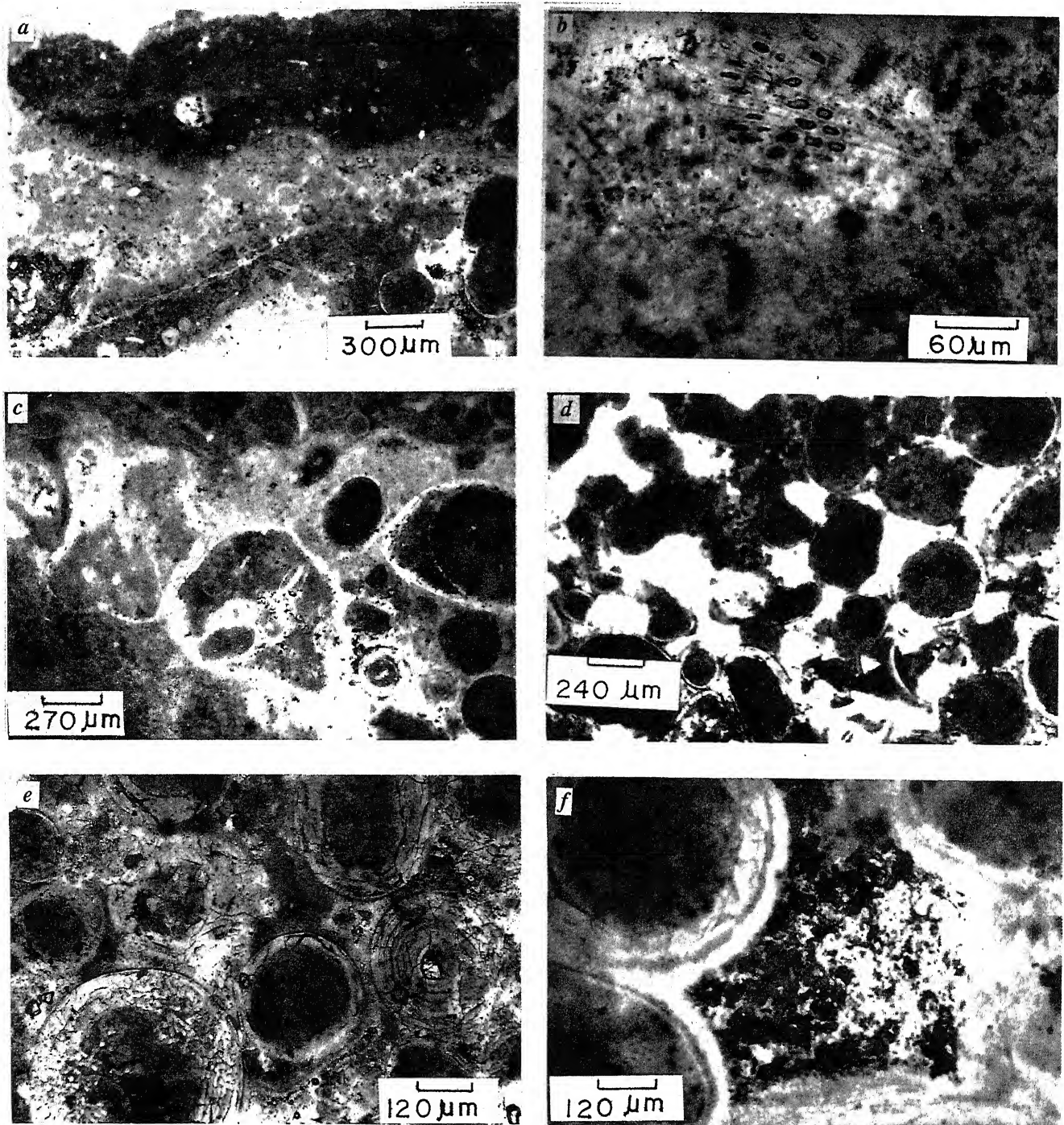


Figure 4 a–c. Photomicrographs (plane polarized light) from micrite-dominated limestone. *a*, showing distinct ferruginised micrite-dominated layer on the top and light brown micrite layer below; *b*, partly micritized algal body; *c*, a micrite patch with cemented intraclasts; *d–f*, photomicrographs from pellet-rich limestones (plane polarized light); *d*, pelletal grains in aragonite cements, porous fabric is due to the boring activity of algae; *e*, oolites which are infected by microbial activity showing obscured laminae; *f*, aragonitic isopachous rim cements, porefilling aragonite cements are being replaced by calcite mosaics and terrigenous clays and quartz in the interstices.

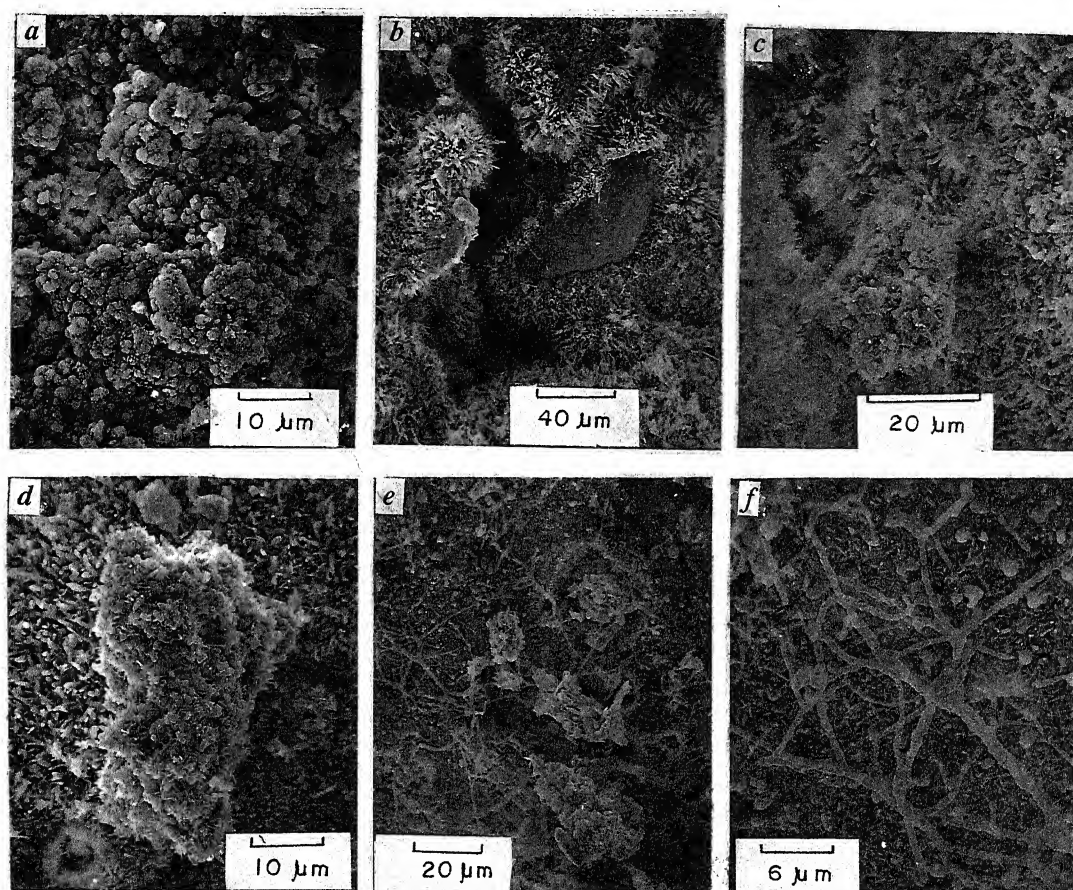


Figure 5a-e. *a*, Micrite matrix showing that it is an aggregate of 1-2 μm size globular particles; *b*, acicular aragonitic fringe cements; *c*, clotted aragonite needles; *d*, a globular calcite aggregate on top of aragonite needles; *e*, globular calcite in boring cavities, several fungal filaments on aragonite matrix can also be seen; *f*, enlarged portion of 'e' showing filaments which are irregularly branched.

supratidal limestones are thicker and laterally more continuous than those in intertidal conditions¹². So the thin, laterally discontinuous micrite laminations (Figure 4a) in some limestones and intraclasts, shell and pellet-dominated sediments with mature oolites in others probably suggest that the sediments were accumulated in intertidal conditions. Detrital material (Figure 4f) most probably washed onto these sands and subsequently filtered down into the pores under agitating conditions.

(i) Endolithic algae/fungal activity is evident in these limestones by the porous fabric (Figure 4d), microbially infected oolitic laminations (Figure 4e), algal borings and fungal filaments (Figure 5e,f). Endolithic algae are abundantly photosynthetic and they bore intensively in nearshore zones¹³.

(ii) Although micrite is abundant in both subtidal and intertidal limestones^{14,15}, it is mostly high-magnesium calcite in submarine cemented limestones¹⁶. The micrite reported here, however, is aragonite.

(iii) Four types of cements exist: thinly laminated micrites (Figure 4a), dense patches of micrite (Figure 4c), acicular aragonite (Figures 4f, 5b) and peloidal cements. These cements are further complicated by subsequent superposition of other cements. Replacement of pore filling aragonites by calcite (Figure 4f), clotted aragonite needles (Figure 5c) and calcite aggregates on top of aragonite needles (Figure 5d) indicate that initially formed aragonitic cements were subsequently replaced by drusy calcites. Similar cement fabrics were reported¹⁷⁻¹⁹ in intertidal limestones and beachrocks. The formation of clotted aragonites and drusy calcites perched on aragonites was attributed to the high degree of super saturation in the pores of sands at intertidal conditions¹⁷. Micrite patches enclosing terrigenous particles suggest that allochthonous micrite filled the boring cavities. These four types of cements cause different textures in the rock (Figure 3b, c), a feature generally observed in beachrocks.

(iv) Submarine cemented limestones consist of aragonite

skeletal components replaced by high-magnesium calcite and abundant peloidal high-magnesium calcite cements^{16,20–22}. In contrast, the limestones studied here contain minor high-magnesium calcite, rare peloidal cements and unaltered skeletal aragonites. In view of (i) to (iv) we suggest that these limestones were cemented at intertidal conditions.

Relationship of terrace limestones with sea-level changes

The age and depth relationship of the limestones suggest that intertidal conditions existed at 130 m depth terrace at about 11,900 years BP. Contrastingly, the glacio-eustatic sea-level¹⁰ was at –90 m at about 12,000 years BP. Three possibilities exist to explain this disparity:

(i) The limestones might have been transported from shallow shelf. This is unlikely as a terrigenous clayey sand zone (15–20 m thick) exists landward of the terrace (Figure 2a, b). The clay in the interstitial pores of the limestones (Figure 4f) may have originated from this clayey sand zone. Moreover, the limestones are from a wide terrace and the constituents in limestones and in unconsolidated sediments of the terrace are similar.

(ii) Incorporation of modern carbon and replacement of aragonite by calcite may give younger ages of limestones than the actual. This is also unlikely as the dates given here were corrected for modern industrial carbon and, calcite content (low and high-magnesium calcites) is <5% and low-magnesium calcite that replaced the aragonite is a small part within the 5% which may not have major influence on the given dates.

(iii) This part of the continental margin may have subsided after the limestones were formed. It is more likely as the terraces occur at depths more than 120 m (eustatic sea-level low during the LGM–18,000 years BP). Vadose diagenetic limestones of Holocene age on the Fifty Fathom Flat^{23,24}, missing data for the period younger than 8,300 years BP from the offshore and tidal mud flats of Early Holocene age at +8 to +10 m above the present sea-level in the coastal Gujarat and Maharashtra^{25,26} probably support neotectonic activity in this region.

The sea-level rise after 11,900 years BP was 130 m on this margin. The eustatic sea-level rise¹⁰ after 12,000 years BP was only 90 m. This suggests that some time after 11,900 years BP, the margin may have subsided by about 40 m. This subsidence could be due to the overburden of Indus-borne sediments accumulated on the continental slope and in the adjacent Arabian Basin as also suggested by Whiting *et al.*²⁷.

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Petrogenesis of granitic rocks of low- to high-grade transition zone of Krishnagiri, Dharmapuri District, Tamil Nadu

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The granitic rocks of low- to high-grade transition zone of Krishnagiri are distinctly grey coloured, exhibiting a clear gneissic banding with predominant tonalite-granodiorite composition. Ubiquitous occurrence of amphibolite enclaves in the granite suggests a genetic link between the two. The rocks are composed of quartz, plagioclase and subordinate amounts of orthoclase and microcline as light coloured minerals with hornblende and biotite as mafics. Chemically, these granitoids belong to moderate- to high-alumina type and metaluminous granite with average A/CNK value being less than unity. Magmatic parentage is discernible from the behaviour of many major and trace elements. Abundances of certain elements like TiO_2 , MgO, Sr and Ni indicate a basic source. REE data support the partial melting of garnet-bearing amphibolite source in the evolution of these granitoids. The I-type characteristics and low Rb/Sr ratios suggest that the amphibolite source is possibly derived from an enriched mantle/lower crust with mafic constituents. The mineralogy and petrochemistry match well with the granites of IAG + CAG affinity.

THE Archaean rocks of India are a complex suite, comprising greenstones, low, and high-grade gneisses and granites with polyphase and polygenetic characters and ages ranging from 3300 m.y to 2500 m.y (ref. 1). On the basis of the extensive study on these rocks, it is now generally understood that they have complicated plutono-metamorphic history, heterogeneity of petrochemical composition, time-space distribution, obliterated original structures due to subsequent deformations and metasomatism. Granitic rocks, constituting a major component of the Archaean, have also undergone the tectonics. Some petrologists², believe that most of the granitic rocks occurring in Precambrian Cratons and Mobile Belts are better indicators of the crustal processes and compositions as these rocks are considered to owe their evolution to conditions of high temperature and abnormal crustal activity prevailing during orogenic events. Therefore, more emphasis is placed on the investigation of the Archaean granitic rocks in recent years to probe into their probable precursors. The studies, so far, carried out on the litho-units of Transition Zone in

Dharmapuri District mainly concentrated on their metamorphic grade of formation³⁻⁶. The present study constitutes a detailed account of the granitic rocks occurring in the transition zone of Krishnagiri.

Field setting

The granitic rocks, occurring in and around the transition zone of Krishnagiri (Figure 1), are distinctly grey-coloured, exhibiting a clear gneissic banding with dominant tonalite-granodiorite composition and subordinate occurrences of granite (s.s.). Mostly, they are medium-grained with occasional fine and coarse-grained varieties. The gneissic banding, often, exhibits contortion in compositional layering which gives rise to small scale close folds with parallel fold axes. Abundant amphibolite enclaves with different shapes and sizes are present which depict a structural pause with the surrounding granitoids and are regarded as possible relics of the older crust⁷. The last phase of the igneous activity in the area is represented by the profuse development of dolerite dykes cross cutting three granitic rocks dominantly in WSW-ENE and WNW-ESE directions. The trend of the foliation, defined by parallel orientation of mafic minerals, is more or less the same throughout and is generally along the NNE-SSW direction swinging frequently to N-S with easterly dips ranging from 30° to 40°.

Petrography

The medium-grained rocks of the area exhibit distinct gneissic banding consisting of alternating bands of leucocratic and melanocratic minerals of quartz, plagioclase and subordinate amounts of potash feldspars in the former and hornblende and biotite in the latter respectively. Based on mineralogy (Table 1), three types could be recognized using IUGS systematics after Streckeisen⁸, namely: (i) tonalite, (ii) granodiorite and (iii) granite (Figure 2). The granitoids fall in the IAG + CAG fields after Maniar and Piccoli⁹. In thin sections, the feldspars are mostly plagioclase (An_{14-32}) with minor amounts of

orthoclase and microcline, the latter minerals are seen more in granite (s.s.). The plagioclase grains are both twinned, generally, on albite-albite law and untwinned. Sericitization of feldspars is noticed in some sections. The orthoclase is occasionally perthitic and microcline is interstitial between quartz and plagioclase grains. Myrmekitic intergrowths are rarely noticed. The mafic minerals include prevalent hornblende ($2V_x = 68-78^\circ$; $Z^c = 17-19^\circ$), followed by biotite which is distinctly pleochroic from brown to greenish brown. The common minor accessories are sphene, apatite and magnetite.

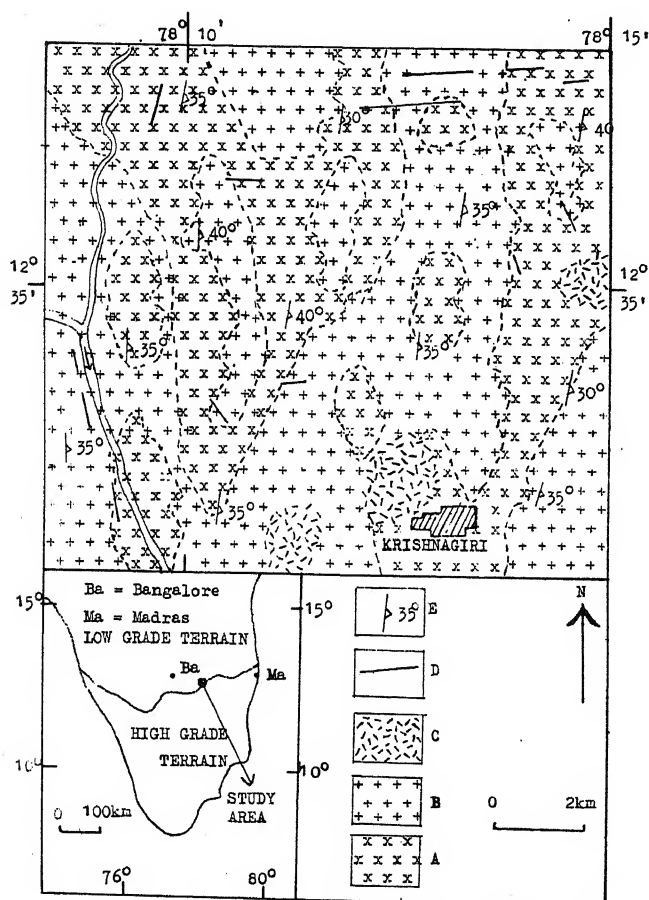


Figure 1. Location and geological map of Krishnagiri. A = Tonalite; B = Granodiorite; C = Granite; D = Dolerite dykes; E = Strike and dip of foliation.

Table 1. Modal composition of the Krishnagiri granitic rocks

Mineral	1	2	3	4	5	6	7
Quartz	23.4	34.4	31.1	32.7	26.9	22.1	26.1
Plagioclase	48.1	42.7	49.4	37.8	43.7	46.2	29.6
K-feldspar	5.5	4.9	3.4	15.5	13.4	20.2	31.3
Hornblende	16.3	11.1	7.8	10.5	9.5	6.3	7.4
Biotite	5.8	6.1	6.8	2.3	5.7	4.1	4.1
Accessories	0.9	0.8	1.5	1.2	0.8	1.1	1.5
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Sample Nos. 1 to 3 = Tonalite; 4 to 6 = Granodiorite; 7 = Granite.

Petrochemistry

Seven representative samples were carefully selected for chemical analyses. Major elements were determined by conventional wet methods and trace elements were determined by atomic absorption spectrophotometer. Rare earth elements (REE) on selected samples were determined by using spark source mass spectrometry¹⁰. The precision ($\pm 5\%$) and accuracy were checked by using standards and by performing duplicate analyses. The chemical data of the granitic rocks of Krishnagiri are given in Table 2. Table 3 presents some diagnostic chemical parameters.

Major elements

From Table 2, it is observed that from tonalite to granite, there is a gradual increase of SiO_2 , K_2O and gradual decrease of MgO , CaO , Na_2O and Sr , while the remaining oxides show fluctuations. In the differentiation index (DI) diagram (Figure 3), with increasing DI, there is an increase in SiO_2 , K_2O , Ba , K/Rb , K/Ba and K/Sr and decrease in Na_2O , MgO , CaO and Sr . All the parameters trace a smooth trend against DI, suggesting that these granitoids are cogenetic. In case of the Ca-Na-K distribution, the granitoids follow a calcalkaline trend¹¹. $\text{Na}_2\text{O}/\text{K}_2\text{O}$, which usually will be less than one in the microcline rich granites, is high and more than one (average 2.18) and seems to be controlled by the abundance of plagioclase in the rocks testifying to their dominant tonalite-granodiorite composition¹². World-wide observations indicate that soda-rich granitoids are of Archaean age and K-rich granitoids are of Proterozoic

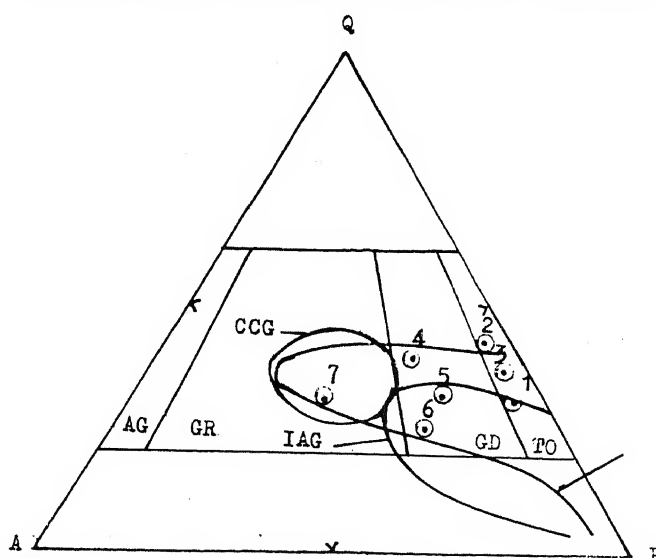


Figure 2. Modal Q-A-P plot using IUGS systematics after Streckeisen⁸. Description of the samples are as given in Table 1. IAG = Island Arc Granites; CAG = Continental Arc Granites; CCG = Continental Collision Granites. The fields are after Maniar and Piccoli⁹.

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and younger age¹³. The tonalite–granodiorite suites of the Shimoga–Honali, Holenarsipur and Chitradurga, having Na₂O/K₂O ratio more than one, have yielded an age older¹⁴ than 2.5 b.y, whereas granitoids of adamellite–granite compositions from Closepet area, with the ratio less than one, are younger¹⁵ than 2.5 b.y.

The granitoids of the area are dominantly metaluminous with A/CNK values being less than unity except for one sample which is slightly peraluminous (average A/CNK and A/NK are 0.95 and 1.68 respectively). The agpaitic index is less than one indicating subsolvus nature of these granitoids. Barker *et al.*¹⁶ and Barker¹⁷

Table 2. Chemical composition of granitic rocks from the Krishnagiri area

	1	2	3	4	5	6	7	Average
SiO ₂	67.10	68.78	68.30	71.35	69.66	70.59	72.08	69.69
TiO ₂	0.32	0.28	0.36	0.35	0.40	0.44	0.30	0.35
Al ₂ O ₃	15.84	15.80	15.01	14.30	15.03	14.72	14.52	15.03
Fe ₂ O ₃	1.10	0.78	0.92	0.85	0.92	0.95	0.68	0.89
FeO	1.98	1.12	1.53	1.31	1.41	1.17	1.29	1.40
MnO	0.10	0.06	0.07	0.08	0.11	0.05	0.09	0.08
MgO	2.22	1.20	1.78	0.87	0.75	0.97	0.62	1.20
CaO	5.07	4.61	4.40	3.74	3.91	3.53	2.01	3.90
Na ₂ O	4.38	4.98	4.45	3.73	3.67	3.71	2.98	3.99
K ₂ O	1.20	1.47	1.53	2.22	2.58	2.79	3.41	2.17
P ₂ O ₅	0.15	0.32	0.27	0.30	0.24	0.29	0.58	0.31
LOI	1.13	0.93	1.37	1.28	0.81	0.98	1.08	
Total	100.59	100.33	99.99	100.38	99.49	100.19	99.64	
<i>Trace elements</i>								
Rb	45	49	52	38	35	57	41	45
Ba	171	215	135	156	145	201	197	174
Sr	312	215	285	185	235	193	172	228
Ni	26	31	21	28	19	23	17	24
Cr	27	25	30	17	19	23	20	23
Y	16	12	22	15	18	19	13	16
Zr	131	165	181	156	154	172	150	158
<i>REE</i>								
La			31	37			41	36
Ce			28	36			31	32
Sm			5.1	4.8			5.7	5.2
Eu	ND	ND	0.95	0.75	ND	ND	1.21	0.97
Tb			0.51	0.64			0.58	0.58
Er			0.41	0.74			0.63	0.59
Yb			0.66	0.72			0.42	0.60

Description of the samples as in Table 1; ND = Not determined.

Table 3. Some diagnostic chemical parameters

	1	2	3	4	5	6	7	Average
DI	67.29	74.74	72.55	76.94	75.33	77.94	82.48	75.32
Na ₂ O/K ₂ O	3.65	3.39	2.91	1.68	1.42	1.33	0.87	2.18
A/CNK	0.89	0.87	0.88	0.92	0.94	0.94	1.18	0.95
A/NK	1.85	1.60	1.67	1.65	1.70	1.60	1.68	1.68
Agpaitic index	0.54	0.62	0.60	0.60	0.59	0.63	0.60	0.60
Ba/Sr	0.55	1.00	0.47	0.84	0.62	1.04	1.15	0.81
K/Rb	222	251	248	489	620	408	700	420
Ca/Sr	117	154	112	145	108	132	85	122
Rb/Sr	0.14	0.23	0.18	0.21	0.15	0.29	0.24	0.21
REE	—	—	66.63	80.65	—	—	80.54	75.94
(LREE/HREE) _N	—	—	10.69	9.44	—	—	12.29	10.81
La _N /Yb _N	—	—	28.47	31.14	—	—	59.16	39.59
La _N /Sm _N	—	—	3.33	4.23	—	—	3.95	3.84
Ce _N /Yb _N	—	—	9.64	11.36	—	—	16.78	12.59
Ce _N /Y _N	—	—	4.34	8.18	—	—	8.19	6.90
Eu/Eu*	—	—	0.66	0.50	—	—	0.82	0.66

Description of the samples as in Table 1.

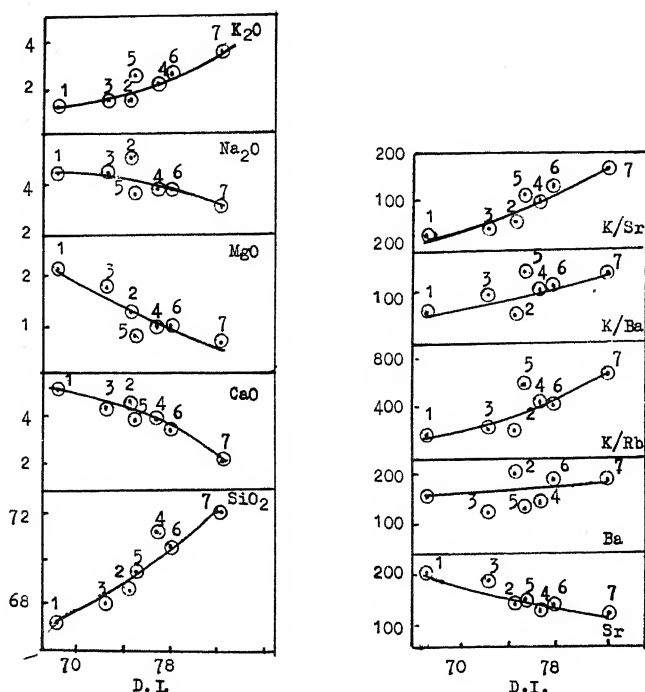


Figure 3. Plots of the major and trace elements against differentiation index (DI), for the granitic rocks of Krishnagiri. Symbols as in Figure 2.

have classified the granitic rocks into high alumina and low alumina types using weight percentages of SiO_2 and Al_2O_3 . Barker¹⁷ has indicated 15% Al_2O_3 at 70% SiO_2 as the dividing point for these two types. Recently, Sarvothaman¹⁸ has suggested, after an elaborate study, that the specifically calculated $\text{SiO}_2/\text{Al}_2\text{O}_3$ ratio depicts an unequivocal demarcation between low- and high-alumina types. The $\text{SiO}_2/\text{Al}_2\text{O}_3$ ratio for the granitic rocks of Krishnagiri ranges from 4.24 to 4.99, thereby assigning them to moderate to high-alumina type.

Trace elements

Barium. Ba shows generally moderate values varying from 135 to 215 ppm averaging 174 ppm. The K/Ba ratio, depicting wide fluctuations from 57 to 150 and averaging 106, shows an increase with increase in DI (Figure 3). Ba/Sr ratios of these granitoids are low to moderate with the average value of 0.81. Similar value is reported for Marginal Gneiss Suite (average 0.97) of Holenarsipur Belt of Karnataka¹⁹.

Rubidium. Rb values in the present granitic rocks show scatter very narrowly from 35 to 57 ppm and correlating with the low potash content of the rocks. The average value of 45 ppm in this suite is fairly comparable to the Kaptipada tonalite–granodiorite suite of Singhbhum Craton (average 60 ppm)⁷ and mean Archaean High alumina tonalite (average 44)²⁰. The K/Rb values

show wide variations from 222 to 700 with the average value of 420.

Strontium. Sr levels in the present case depict appreciable fluctuations (from 172 to 312 ppm) showing depletion towards higher SiO_2 levels and sympathetic with behaviour of Ca. The values are (average 228 ppm) correlating well with the Ca content (average 2.80) of this suite. The average Rb/Sr ratio of 0.21 is closely comparable to those of Phases I and II of Singhbhum granitoid with tonalite–granodiorite composition (average 0.21)²¹, of tonalite–granodiorite suite of Kaptipada (average 0.19)⁷ and of equigranular granitoid suite of the Bonai Pluton (average 0.21)²².

Nickel. Ni content of this suite ranges from 17 to 31 ppm with an average value of 24 ppm, which is fairly similar to the concentrations reported for the trondhjemites of Hassan District (average 19 ppm)²³ and for tonalites of Dharwar Craton (average 16.5 ± 4.9 ppm)²⁴.

Chromium. Cr values, varying from 17 to 30 ppm, show decrease towards increase in SiO_2 .

Yttrium. The granitic rocks of the area show lower values of Y ranging from 12 to 22 ppm averaging 16 ppm. Y_N depicts negative correlation with Ce_N/Y_N , which probably suggests the role of garnet in the formation of granitic precursors²⁵. A positive correlation is seen between Y and TiO_2 (Figure 4).

Zirconium. The concentration of Zr shows limited range of variations from 131 ppm to 181 ppm averaging 158 ppm, which is moderately low like other Archaean gneisses²⁰. Similar to Y, Zr also exhibits a linear correlation with TiO_2 (Figure 4).

Rare earth elements. The granitic rocks show narrow ranges in REE abundances (66.63–80.65 ppm). Ce_N/Yb_N ratio ranges from 9.64–16.78 averaging 12.59. La_N/Yb_N ratio exhibits very high values ranging from 28.47 to 59.16, whereas, La_N/Sm_N values show a limited range of variation (3.33–4.23).

The chondrite normalized REE patterns in these granitoids show a highly fractionated nature with a conspicuous LREE enrichment and HREE depletion (Figure 5). Generally, they show slight to moderate negative Eu anomaly with Eu/Eu^* ratio ranging from 0.50 to 0.82.

Discussion and conclusion

The calc-alkaline affinity of the grey granitic rocks of the area suggests their emplacement under thick continental crustal conditions indicating greater temperatures in the lower crust, a situation capable of generating

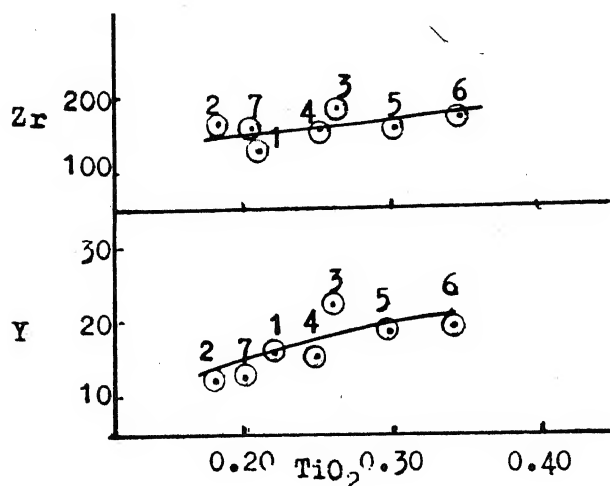


Figure 4. TiO_2 versus Y and Zr diagram. The samples show a linear correlation between the elements. Symbols as in Figure 2.

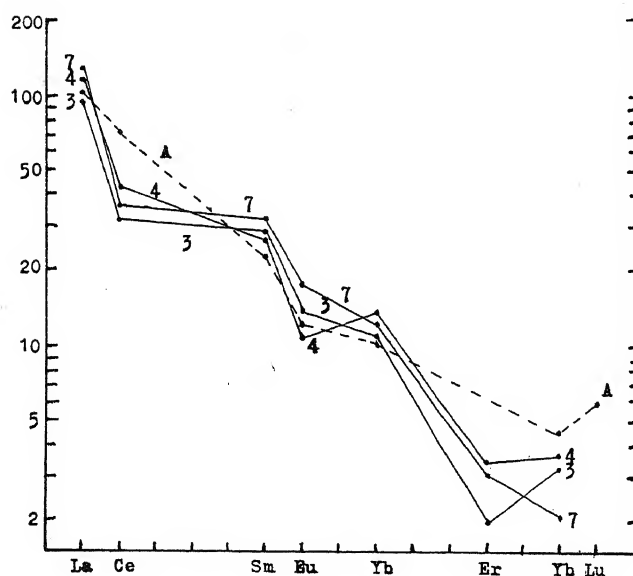


Figure 5. Chondrite normalized REE abundances of granitoids of Krishnagiri. Description of the samples as in Figure 2. A = sample no. KP-5 of Kaptipada⁷.

large volumes of tonalitic magmas. The phenomenon appears characteristic of early stages of many magmatic cycles recorded in shields^{17,26-28}. Many workers^{11,20,28} advocated different models for the origin of calc-alkaline orthogneisses. They are:

- the nature of source rocks – various mantle compositions, gabbros, wet basalts, amphibolites, simatic and sialic granulitic crust, greywackes, other sediments, etc.
- the process – partial melting and fractional crystallization of variable nature and degree involving garnet, amphibole and/or plagioclase as residual or fractionating phases.

The general high content of certain oxides like TiO_2 , MgO and trace elements such as Sr and Ni in these granitoids suggests the presence of mafic components in the source region²⁹. The higher values of these elements reflect the characteristics of a magma evolving probably from an amphibolite-bearing source. A similar source has been invoked for the granitic rocks of India^{7,23,30}. The amphibolite enclaves are considered to be relics of a basaltic crust and suggest as a source for the tonalite–granodiorite suite of Nuk Gneiss³¹. Amphibolite, occurring as enclaves, has been regarded as the source for the various tonalite–granodiorite suites of India^{7,22,23}.

The highly fractionated REE patterns with high LREE/HREE ratios suggest the role of hornblende/garnet in the source rocks of Krishnagiri granitoids^{32,33}. The moderate to high Ce_N/Y_N ratios (4.34–8.19) imply that Y is retained in residual garnet³⁴. Positive correlation of Y and Zr with TiO_2 , negative correlation between Y_N and Ce_N/Y_N and high LREE/HREE ratio are in conformity with equilibration between melt and solid that is controlled generally by garnet³⁵⁻³⁷. Also granitoids which are considered to have residual garnet in their partial melt have Ce_N/Yb_N ratio³⁸⁻⁴⁰ more than 10. This ratio in the granitoids of the area ranges from 9.64 to 16.78, supporting the presence of garnet in the residual melt.

Possible mechanisms have been explored, regarding the process of formation of the granitic rocks of Krishnagiri. The subordinate occurrence of granite (s.s.) in the present area precludes the formation by ensialic anatexis. The absence of petrographic features like albitization of plagioclase and replacement of plagioclase by potash feldspar rule out the process of metasomatism as well. Also the model of fractional crystallization is set aside as there is distinct absence of intermediate rocks, like syenites and/or diorites and also there is no continuous order of basic to acidic rocks except for quartz-rich granites (s.l.). The most appropriate mechanism appears to be partial melting of basic rocks. It has been envisaged that the formation of cumulates during the fractional crystallization of a basic magma of mantle derivation emplaced into the lower crust in a tensional tectonic environment liberates hidden heat which brings about partial melting in the lower crust^{16,41}. Ellis and Thompson⁴², after conducting partial melting experiments, have observed that a suite of calc-alkaline rocks can originate by melting of amphibolites or basalt (or similar basic rocks) and that during the partial melting of basic rocks, say at 1000°C and one atmosphere pressure, the melts produce metaluminous granites and are, therefore, diopside normative, whereas at 5 kb pressure range, basic rocks may give rise to melts of metaluminous granites at temperatures far below 1000°C . The granitic rocks of the area are dominantly metalu-

Table 4. Comparative statement of some diagnostic parameters of granitoids of Krishnagiri and Kaptipada (south-eastern part of Singhbhum Craton, Orissa)

Parameter	Krishnagiri area	Kaptipada area
Field association	Ubiquitous amphibolite enclaves present	Presence of dominant amphibolite enclaves
Type of granitoid	Dominant tonalite–granodiorite	Tonalite–granodiorite
Mineralogy	Qz + plag. + K-feld. + hornb. + biotite	Qz + plag. + K-feld. + hornb. + biotite epi + chlorite
I- or S-type	I-type	I-type
Petrochemistry		
K ₂ O/Na ₂ O	0.59	0.50
CaO/Na ₂ O + K ₂ O	0.64	0.60
TiO ₂	0.35	0.43
Rb/Sr	0.21	0.19
REE	66–80	67–101*
LREE/HREE	High	High*
Eu/Eu ⁺	0.50–0.82	0.70†

*Sample nos of Kaptipada KP-1, KP-2 and KP-5 (Table V)⁷.

†Inferred from the sample no. KP-5 (Table V)⁷.

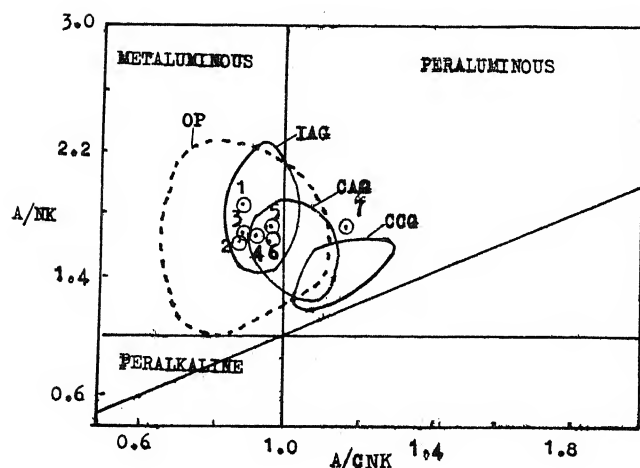


Figure 6. A/NK versus A/CNK binary plot. IAG = Island Arc Granites; CAG = Continental Arc Granites; CCG = Continental Collision Granites; OP = Oceanic Plagiogranites. Fields are after Maniar and Piccoli⁹. Symbols as in Figure 2.

minous with the average ASI value less than unity. In view of the above contention, the process of partial melting of amphibolite source appears to be more tenable in the evolution of the granitic suite in this area. The same process has been attributed to the origin of similar granitic rocks in several parts of Peninsular India^{7,23,33}.

Several occurrences of I- and S-type granites are reported in the Indian shield by Dhanaraju *et al.*¹³. Most of the following features indicate I-type characteristics^{13,43,44} for the granitoids in this region: (i) high Na₂O content, (ii) average molar A/CNK ratio is 0.95, indicating metaluminous nature, (iii) average molar A/NK ratio is 1.67, (iv) dominant diopside normative, (v) regular inter-elemental behaviour, (vi) biotite and hornblende as mafic minerals, (vii) ubiquitous amphibolite enclaves and (viii) presence of sphene and apatite as common accessories.

Geochemical signatures in granitic rocks are controlled by their source characteristics⁴⁵. These granitoids might have inherited the petrochemical features probably from an 'enriched' mantle or lower crust, with mafic constituents^{29,30}. The above assumption is supported by the high abundances of certain elements in these granitoids as mentioned earlier and I-type lineages with lower Rb/Sr ratio. The I-type granitoids are genetically related to the mantle source⁴³. Similar source characteristics have been invoked for the tonalite–granodiorite suite of the Kaptipada area having the same petrological features⁷ as that of the granitoids of the present area (Table 4).

Maniar and Piccoli⁹ have devised a number of discriminant diagrams using major element chemistry in order to know the geotectonic environment in which granitic rocks are emplaced. In the diagram employing A/NK versus A/CNK ratios, a clear demarcation of Island Arc Granites (IAG), Continental Arc Granites (CAG) and Continental Collision Granites (CCG) could be made effectively. In the binary diagram employing the above ratios (Figure 6), the granitic rocks of the Krishnagiri area plot themselves in IAG + CAG field, which has already been attested in the modal diagram (Figure 2). The IAG + CAG granites have A/CNK values less than 1.15, which is highlighted in the present granitic rocks. The authors⁹ further rule out the possibility of discriminating between IAG and CAG based on the major elements.

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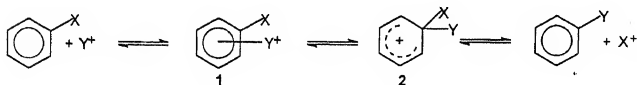
Possibility of proton oscillations through the benzene ring

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The possibility of proton oscillations above and below the plane of the benzene ring, through its centroid, in protonated benzene is pointed out, in this communication.

PROTONATION of benzene and other aromatic hydrocarbons has been studied over the years by a variety of methods¹. The nature of the arenium ion that is formed as the transition state in an electrophilic aromatic substitution has been the focus of attention of a number of experimental and theoretical studies (for example, see ref. 2 and references therein). It is generally believed that a π -complex (1) is formed first and then it rearranges into a σ -complex (2) before it results in products.



When $X=Y=H$, there is an interesting possibility: the proton in (1) could lie either above or below the plane of the benzene ring and it could undergo an oscillatory motion between the two positions, through the centre of the ring.

In order to examine such a possibility we have computed the $H^+-C_6H_6$ interaction energy keeping C_6H_6 in its equilibrium geometry³ ($r_{C-C}=1.386$ Å, $r_{C-H}=1.076$ Å) and varying the distance (Z) of H^+ from the centre of the C_6H_6 plane by the LCAO-MO-SCF approach using the 6-311G basis set and the Gaussian 94 set of programs⁴.

It is clear from the results shown in Figure 1 that there is a potential well of depth of 4.38 eV, with the minimum at $Z=\pm 1.1$ Å and that we are dealing with a stable ionic species that is likely to be found in nature. Mulliken population analysis reveals that there is substantial charge transfer between C_6H_6 and H^+ at such short distances.

Geometry optimization calculations reveal that even when the proton is at the centre of the ring, the ring is only slightly enlarged ($r_{C-C}=1.424$ Å; $r_{C-H}=1.068$ Å) and that the energy difference between the distorted and the undistorted geometries is only 0.23 eV, implying that the proton can go through the centre of ring. It is worth emphasizing that the energy of the $C_6H_7^+$ ion with

an H in the geometric centre of the benzene ring is well below (-1.8 eV) that for the separated $C_6H_6 + H^+$ and that there are several bound states corresponding to the oscillatory motion above and below the plane of the ring as can be seen from Figure 1. These bound states were computed using the Fourier grid Hamiltonian approach⁵. A close examination of the figure suggests three types of oscillations. For $0 \leq V \leq -1.8$ eV, there would be 'free' oscillation of the proton between the two sides of the ring. For energies just below the barrier (-1.8 eV), there is the possibility of tunneling. For energies well below the barrier, the proton would oscillate about the equilibrium position on either side.

In order to make sure that our findings are not dependent on the quality of the basis set, we have computed the entire potential-energy curve shown in Figure 1 using 6-311G** basis set, which includes polarization functions. While the depth of the well and the height of the barrier in between change in magnitude with the basis set, the major features of the double well potential remain the same.

It is worth pointing out that several semiempirical and *ab initio* calculations (for example, see ref. 6) on $C_6H_7^+$ have shown that the σ complex (2) is more stable than the π complex (1), in the context of electrophilic aromatic substitution. But that is not quite relevant to the present investigation which focuses attention on the penetration of the ring by a proton.

The possibility of the charge transfer ($C_6H_6 + H$) channel suggests that the proton undergoing oscillatory motion between the two sides of the benzene ring may not stay attached to the ring forever. The lifetime of the species would depend on the crossing (or avoided crossing) between the potential energy curves for $C_6H_6 - H^+$ and $C_6H_6^+ - H$ interaction. Investigation of this aspect of the problem is presently being taken up. Regardless of the nature of the crossing between the two curves, the

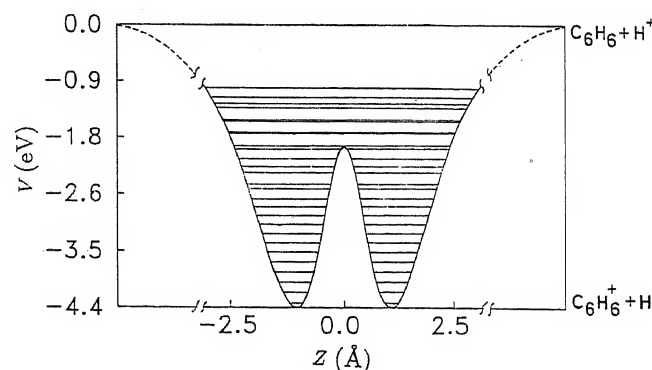


Figure 1. The interaction potential for $H^+-C_6H_6$ with the zero of energy corresponding to the asymptotically separated H^+ and C_6H_6 in its equilibrium geometry. For reference, the energy of $C_6H_6^+ + H$ state is included in the figure.

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proposed structure can be expected to play an important role in proton-benzene collisions⁷. Also, under conditions as in a mass spectrometer or in interstellar clouds⁸ $C_6H_7^+$ could live for a sufficiently long time and transitions between its electronic states and also between the bound states supported by the double well potential could account for some of the hitherto unaccounted emissions, particularly in the long wavelength regions.

Preliminary calculations⁹ show that there are no significant minima but there exist barriers to penetration through the ring in the interaction of C_6H_6 with H, H⁺ and He, thus making H⁺ a unique partner in exhibiting such an oscillatory motion.

If such a motion could exist in protonated benzene, it could exist in a variety of other protonated aromatic hydrocarbons such as naphthalene, anthracene, etc. We have indeed found this to be the case⁹.

There has been a lot of interest in collisions of neutrals and charged species with fullerenes and the possibility of trapping atoms/ions inside the cage¹⁰. In this context the possibility of proton motion through the ring becomes quite relevant.

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Nucleotide frequency map: A new technique for pictorial representation of dinucleotide frequencies

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In this communication, a method of presentation of dinucleotide frequencies in the form of a contour diagram (map), designated as dinucleotide frequency map (DNFM), has been used for the first time to analyse the compositional bias of different nucleic acid sequences. Such maps provide a method of visualization of the nucleotide usage at a glance and allow simultaneous representation and comparative analysis of multiple sequences of different origins. Using the technique of DNFM, it has been shown that the dinucleotide frequency distribution profile of any nucleotide sequence often exhibits distinct statistical bias, which is not predictable from the knowledge of its base composition. Analysis of bacterial rDNA operons showed that 16S and 23S rRNA genes of such species, in general, follow similar dinucleotide patterns, which are different from those of the intervening regions. The technique of DNFM has also been applied to analyse the compositional heterogeneity of the genomic sequence of the bacteriophage lambda to show that the dinucleotide frequencies vary along the phage genome depending on the distribution of open reading frames.

COMPOSITIONAL heterogeneity is an intrinsic feature of natural nucleic acid sequences. At the genome level, eukaryotic and prokaryotic sequences exhibit hierarchy in the frequencies of appearance of most dinucleotides¹. Instances of distinct bias in dinucleotide usage in genomic sequences include underrepresentation of T_pA and overrepresentation of G_pC in most temperate bacteriophage sequences², C_pG suppression in vertebrate non-oncogenes^{3,4}, animal mitochondrial genomes and many thermophilic bacterial sequences² and abundance of G_pG/C_pC in animal mitochondrial genomes and chloroplast genomes⁵. A revealing contrast in statistical composition is often observed for long versus short DNA sequences, primarily because of the presence of local signals² such as promoter, enhancer and termination signals, or genetic mosaicism resulting from horizontal gene transfer, transposition or recombination events and also due to the fact that coding and non-coding regions of any sequence, in general, have distinct bias in short oligonucleotide distributions^{6,7}. Interpretation to such compositional heterogeneity usually centres on structural or conformational preferences², context-dependent mutational events^{8,9}, methylation effects^{2,10} processes of rep-

lication and repair^{8,9}, etc. Nucleosome positioning interactions with DNA-binding proteins and ribosomal binding of mRNA are known to be strongly affected by dinucleotide arrangements^{11,12}. It has recently been suggested² that conformational stacking arrangements are principally determined by dinucleotide configurations. Replication error rates, including nucleotide misincorporation and transient misalignment, have also been shown to be context-dependent^{8,9}.

Realizing the importance of compositional analysis of nucleic acid sequences, a series of analytical computational and graphical techniques have been developed^{1-5,7,13-15}. However, a major problem of nucleotide usage analysis is the involvement of a large number of variables. Even a simple comparative analysis of the dinucleotide usage in different DNA/RNA sequences involves handling of two or more sets (for pairwise comparison or simultaneous comparison of multiple sequences), each having 16 different numbers. For trinucleotide frequency analysis, the number is four times higher. Both the qualitative and quantitative analyses of such a large number of variables would practically be impossible unless a suitable method for visual representation of these numbers is developed. It is in this context that the present report describes a technique used for the first time for pictorial representation of the dinucleotide frequencies of any nucleic acid sequence using contour maps, which will enable quick visual comparative assessment of the data at a glance. These maps, designated as dinucleotide frequency maps (DNFMs), have been used to analyse the compositional bias of different sequences.

The basic formalism for construction of DNFMs is as follows: The percentage of occurrence of sixteen dinucleotides in the query sequence has been plotted along the lines joining the centre of the regular sixteen-sided polygon and its vertices, where each vertex corresponds to a particular dinucleotide and appropriate maximum and minimum values of the dinucleotide frequencies are assigned to the vertices and the centre respectively. All the consecutive points plotted along different axes are joined to get the DNFM of the sequence. More than one sequence can be mapped on the DNFM following the same formalism.

It is worth mentioning at this point that sixteen dinucleotides can be arranged in $(15!)/2$ different ways

(! means factorial). By several trials it has been found that the order of presentation as per the first two bases in the codons, as they appear in the tabular form of the genetic code, provides an attractive visual impact, even for most of the non-coding sequences (such as rDNA sequences) so far examined by our group.

The technique has been applied to analyse the compositional bias that might exist in different nucleotide sequences, both at the gene and genome levels. The question often raised in dinucleotide usage studies is whether the di-, tri- and higher-order nucleotide distributions of any sequence are merely the consequences of its base composition or do they truly reflect preferences for certain sequence patterns. To address this issue, sequences of small subunit rRNA genes of tomato, strawberry, *Leishmania donovani* and *Leishmania major* having similar base compositions (Table 1) were taken from the database and a random sequence having the average length and base composition of these sequences (Table 1) was generated through first-order Markov Chain simulation. The latter sequence was taken as a control, as it should have the dinucleotide compositions expected from its mononucleotide frequencies. Figure 1 shows the DNFMs of these rDNA sequences along with the control sequence. While the rDNA sequences from the plants (red and yellow) exhibited similar DNFMs, those from the parasites (blue and pink) produced a visibly different map. None of the DNFMs follows the trajectory exhibited by the random sequence (green) having similar base composition. In all the four rDNA sequences examined, the percentage of occurrence of the dinucleotides G_pC , G_pT , A_pG and T_pA was significantly lower and that of the dinucleotides T_pT , G_pG and A_pA was higher than that expected from their mononucleotide composition. In the plant sequences, A_pT was overrepresented, while the parasite sequences exhibited an abundance in C_pA . There were many such instances which suggest that the dinucleotide distribution in a sequence is not a consequence of its base composition.

To examine the extent of compositional homogeneity in small segments (say, an operon) of the genome(s) of a species, the DNFMs of different segments of the rDNA operons of different bacterial species have been generated. The DNFMs of 16S and 23S rRNA genes of *E. coli* and their intervening spacer region are shown in Figure 2. In most of the cases examined so far, the

Table 1. Mono-nucleotide frequencies

Occurrence of (%)	Organism				
	<i>L. esculentum</i> (tomato)	<i>F. ananassa</i> (strawberry)	<i>L. donovani</i>	<i>L. major</i>	Average
A	24.89	25.00	25.03	25.08	25.0
T	25.55	25.72	25.21	25.12	25.4
C	22.11	22.06	23.04	23.11	22.6
G	27.44	27.22	26.71	26.67	27.0

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DNFMs of 16S and 23S rRNA genes follow almost similar contours, while that of the spacer region (usually including a couple of tRNA genes) exhibits a distinct trajectory (Figure 2). Thus, the small and large subunit rRNA genes, though generally do not have significant sequence homology, follow similar dinucleotide distribution patterns, which are distinctly different from those of the intervening regions.

The technique of DNFM may also be applied to analyse the global distribution of dinucleotides in genomic sequences. The circular genomic sequence of lambda, a linearized version of which is available at the GenBank, has a striking feature concerning the distribution of the coding regions along its two strands. For the first 20,000 and last 8,000 bases (approx.) of the genome (total length 48,502 bases), all the known and putative open reading frames (ORFs) are found to occur in the reported strand, while all ORFs in the region starting from 20,147 to 37,940 bases are in the complementary strand. To examine whether dinucleotide distribution pattern is similar throughout the genome, or it differs between coding and non-coding region, the genomic sequence was divided into different parts and the DNFM of each segment was generated (Figure 3). The DNFMs of the two parts from the first 20,000 bases of the genome (representing the coding strand) follow similar contours except small deviations along a few axes (pink and blue), but those from the middle portion of the genome (representing sequences complementary to the coding region), though resembling one another (green and red), are quite distinct from those

of the coding strand. Both the sequences from the coding part of the genome exhibit abundance in G_pC , G_pA , G_pG and C_pG , while the dinucleotides T_pT , T_pC , T_pA and A_pT are overrepresented in the non-coding region of the genome. Similar results were obtained when the sequence was divided into segments having smaller lengths. This indicates that the dinucleotide usage patterns vary along the genome of lambda depending on the distribution of ORFs. However, when the DNFM of the complementary sequence of the middle portion (20,147

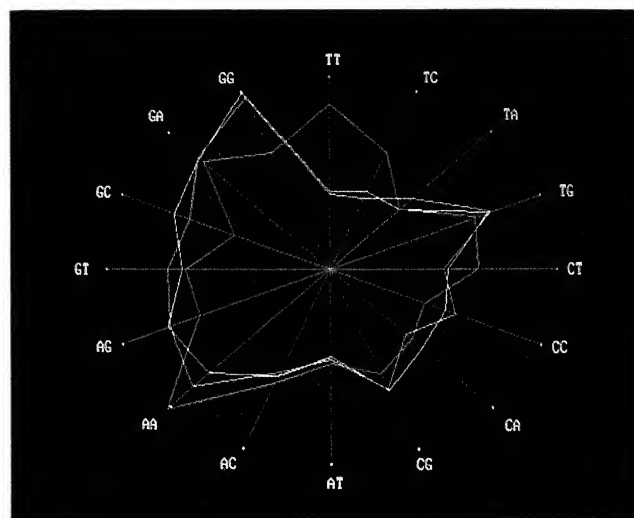


Figure 2. Comparison of 16S, 23S rRNA genes and their intervening spacer region of *E. coli* (V00348). Pink: 16S rRNA gene; green: 23S rRNA gene; blue: intervening spacer region. The centre and each vertex represent respectively 0 and 10% occurrence of the dinucleotides.

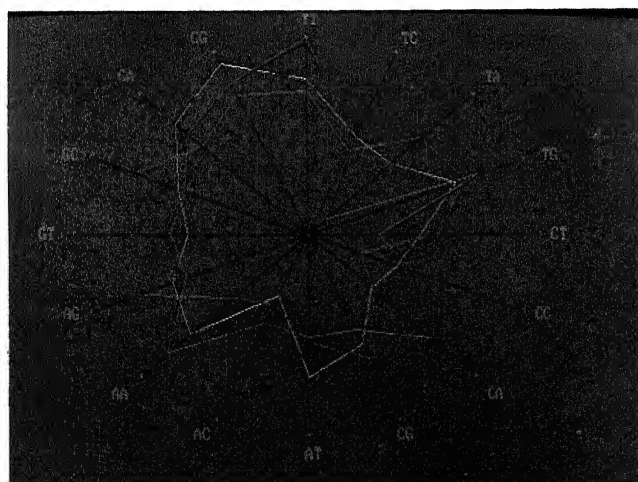


Figure 1. Dinucleotide frequency maps of small subunit rRNA genes. Yellow: *L. esculentum* (tomato) ($\times 13828$) 1800 bases; red: *F. ananassa* (strawberry) ($\times 15590$) 1804 bases; blue: *L. donovani* ($\times 07773$) 2205 bases; pink: *L. major* ($\times 53915$) 2137 bases; green: random sequence having average base composition of the above four sequences as mentioned in Table 1, 2000 bases. The centre and each vertex represent respectively 3 and 8.5% occurrence of the dinucleotides.

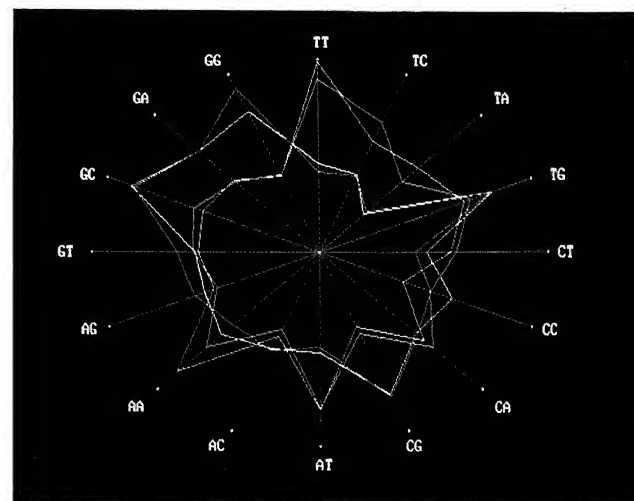


Figure 3. Dinucleotide frequency maps of different parts of sequences of Lambda genome (V00636). Pink: bases 1-10000; blue: bases 10001-20000; green: bases 20151-29151; red: bases 29152-37652. The centre and each vertex represent respectively 0 and 10% occurrence of the dinucleotides.

to 37,940) of the genome, which represents the actual coding sequence, was generated the pattern did not show similarity to those of the coding sequences in the first 20,000 bases of the genome (data not shown). Thus all the coding regions of the genome do not exhibit similar compositional bias.

The compositional heterogeneities of nucleotide sequences at isochore level were analysed by Karlin *et al.*² using dinucleotide relative abundance values, which assess the contrast between observed dinucleotide frequencies and those expected from the component mononucleotide frequencies. A differential map plotting such dinucleotide relative abundance values can be constructed following a formalism similar to that reported here. However, such differential maps will be statistically significant only for long contigs of DNA sequences (> 100 kb) and hence will not be suitable to analyse the local bias in sequences. This formalism may be extrapolated to map the trinucleotide frequencies of any sequence, in which the map will have sixty-four axes instead of sixteen.

It has already been reported that compositional analysis of nucleotide sequences can be used to address various problems such as discrimination between introns and exons of eukaryotic sequences⁷, presence of local bias in dinucleotide compositions in regulatory regions of the genome (promoter, enhancer, etc.)², assessment of the coding potential of putative ORFs in uncharacterized DNA sequences, etc.⁶. However, all these studies involve a large number of numerical variables and the technique described here might be useful for simultaneous pictorial representation and visual comparison of such variables. Some of these problems are currently under study in our laboratory.

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Differences in energy metabolism in cells of neuronal and glial origin – ³¹P NMR spectroscopic study

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³¹P NMR spectroscopy has been used to study the energy metabolism in PC12 and U-87MG cells of neuronal and glial origin respectively. Both types of cells exhibited the resonances arising from adenosine triphosphate, inorganic phosphate and phosphomonoester. In addition, phosphocreatine was found to be present in PC12 cells whereas in U-87MG cells a prominent resonance due to diphosphodiester was observed. The pathways of ATP production were investigated using specific inhibitors of glycolysis and oxidative phosphorylation. Both glycolysis and oxidative phosphorylation pathways contribute to ATP production in PC12 cells, whereas oxidative phosphorylation was found to be the major ATP producing pathway in U-87MG cells. These results indicate that the energy-producing pathways in the cells of neuronal and glial origin could be different.

ADENOSINE TRIPHOSPHATE (ATP) serves as a primary currency of cells, fuelling cellular processes from macromolecular synthesis to signal transduction. It is well known that ATP production is tightly coupled to its consumption. The viability of cells depends on their energy utilization and it is probable that cell death, which is related to a breakdown in membrane function and/or inhibition in macromolecular synthesis, is produced by a local decrease of ATP below a critical level. This suggests that a large and unfavourable perturbation in the energy pathways could be responsible for an impaired cellular function. An impairment in the energy metabolic pathways of the affected cells or tissues has been observed in many neuronal disorders¹⁻⁶. Very often, changes in the normal functioning of cells have been shown to be affected due to the alterations in the energy utilization pathways⁷⁻¹¹. A detailed understanding of cel-

lular energy metabolism is, therefore, vital. NMR spectroscopy is ideally suited for investigating the cellular energy metabolism of intact cells¹²⁻¹⁶. ³¹P NMR spectroscopy of cells provides quantitative information about the various phosphorylated metabolites produced in the energy production and utilization pathways. In addition, intracellular pH and phospholipid metabolism can be studied using ³¹P NMR spectroscopy. In the present study, the energy metabolism in cells of neuronal (PC12) and glial (U-87MG) origin has been investigated using ³¹P NMR spectroscopy.

Eagle's minimum essential medium (EMEM) and RPMI-1640 were purchased from Hi-media, Bombay (India). Foetal calf serum was obtained from Biological Industries, Israel. Bovine and horse serum were prepared in the laboratory. Sodium pyruvate, glutamine, glucose, N-2-hydroxy methyl piperazine-N-2 ethane sulfonic acid (HEPES), deuterium oxide (D₂O) and 2-deoxy-D-glucose (2-DG) were purchased from Sigma (USA) while sodium azide was obtained from Loba Chemie Indo Astranal Co., Bombay (India). All other chemicals were procured from various commercial sources and were of analytical grade.

PC12, a rat pheochromocytoma cell line, was obtained from National Tissue Culture Facility (NTCF), Pune (India) and U-87MG, a glioblastoma-astrocytoma cell line, was procured from American Type Culture Collection (ATCC), Rockville, MD (USA). Cells were grown in Nunc plastic tissue culture flasks or in glass bottles at 37°C under CO₂ environment. For PC12 cells, the growth medium consisted of RPMI-1640 with 10% horse serum, 5% foetal calf serum, 20 mM Hepes, 2 mM glutamine, 10 mM sodium bicarbonate and antibiotics. The growth medium for U-87MG cells consisted of EMEM with 5% foetal calf serum, 5% bovine serum, 10 mM Hepes, 1 mM sodium pyruvate and antibiotics. The cells were harvested at 120 h, centrifuged at 1500 rpm for 10 min and resuspended in HBSS containing glucose.

Cells were embedded in an agarose gel matrix in the form of threads so that the cells were held stationary in the NMR tube. Cell embedded gel threads were prepared by the method of Lyon *et al.*¹⁷. Briefly a solution of low gelling temperature agarose (2.6% w/v) was prepared in Hank's balanced salts solution (HBSS) at 50°C. Subsequently, the agarose solution was cooled to 37°C and 1 ml of this solution was mixed with 1 ml of cell suspension so that a typical sample contained 200 × 10⁶ cells/ml. The gel matrix was then extruded under mild pressure through a coil of teflon tubing (0.5 mm i.d.) chilled in an ice-water bath at 10°C. The cell embedded gel threads were collected directly into 1 ml HBSS at the bottom of a 10 mm NMR tube. The 10 mm Wilmad screw cap NMR tube was modified to allow the perfusion of cells. A perfusion insert was fabricated with an inlet and outlet tubing. The insert was fixed on top of the NMR tube after the gel threads

were collected. The cells embedded in the gel threads were perfused with balanced salt solution using a peristaltic pump with a flow rate of 1 ml/min.

³¹P NMR measurements were performed on a Bruker AMX-400 NMR spectrometer with wide bore using 10 mm broad band probe. Spectra were acquired with a field frequency D₂O lock and without proton decoupling. Magnetic field homogeneity was adjusted by maximizing the D₂O free induction decay. ³¹P NMR spectra were obtained at 161.98 MHz using a 15 μs excitation pulse and 1 sec delay time. Chemical shift was measured relative to phosphoric acid and the measurements were performed at 22°C. ³¹P NMR experiments have been performed 3-5 times for both PC12 and U-87MG cells from different passages. The results were reproducible, however, the ³¹P NMR spectra shown in Figure 1 are from typical experiments.

Cell viability was measured using trypan blue dye exclusion method after embedding the cells in the agarose gel threads and again after the NMR experiments were completed. 90-95% cells were found viable at the end of the NMR experiments. This clearly indicates that the cells were metabolically active during the NMR measurements.

Figure 1 shows the ³¹P NMR spectra of PC12 and U-87MG cells from typical experiments. Both PC12 and U-87MG cells showed resonances arising from ATP, P_i and phosphomonoesters (PME). PC12 cells also showed a resonance due to phosphocreatine (PCr) which was absent in U-87MG cells. On the other hand, U-87MG cells showed a pronounced resonance due to diphosphodiester (DPDE), which was relatively small in PC12 cells. Since PCr is produced by the enzymatic reaction involving creatine kinase (CK), it is probable that the

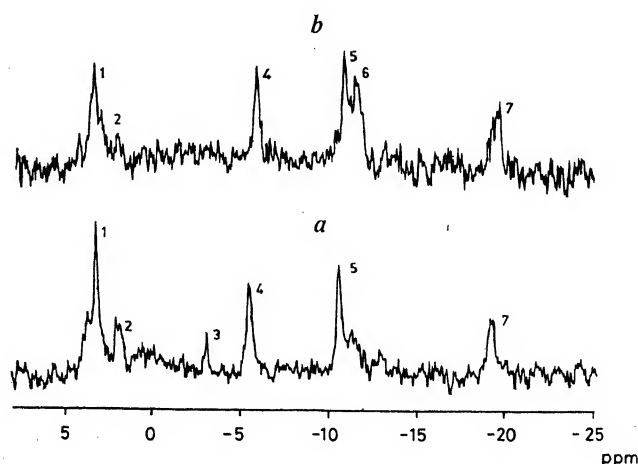


Figure 1. ³¹P NMR spectra of PC12 and U-87MG cells embedded in agarose gel threads and perfused with HBSS containing glucose during the NMR experiments. *a*, PC12 cells and, *b*, U-87MG cells. Spectra were collected 1 h after casting the gel threads. Peaks are assigned as: (1) PME; (2) P_i; (3) PCr; (4) γ-ATP; (5) α-ATP; (6) DPDE and (7) β-ATP.

absence of PCr signal in ^{31}P NMR spectrum of U-87MG cells could be due to the impaired CK activity in these cells. It is also possible that PCr may be present in U-87MG cells in the bound form and therefore is inaccessible to the detection by NMR technique. Holtzmann *et al.*¹⁸ have proposed that in the brain PCr is present in at least two different compartments, one is labile and thought to be present in neuronal cells and the other is stable and is present in astrocytes. The DPDE resonance observed in ^{31}P NMR spectra may arise from the uridine-diphosphosugars, in particular from uridine diphosphoglucose (UDPG). When glucose enters the cells, it may be metabolized to yield energy or it may be stored as an energy reserve in the form of glycogen. The synthesis of glycogen involves the formation of UDPG. The DPDE peak observed in ^{31}P NMR spectrum of U-87MG cells indicates the presence of glycogen reserves in these cells. Glycogen granules are known to be characteristic of astrocytes¹⁹. The PME peak, which was observed in both the cell types, may arise from phosphoethanolamine, phosphocholine and sugar phosphates in the cells¹⁵.

The peak positions of ATP and P_i were almost the same in both types of cells. The same position of P_i (1.9 ppm) in both types of cells suggests that the intracellular pH of these cells is almost similar. The intracellular pH was calculated using the equation¹¹.

$$\text{pH}_i = 6.66 + \log(\text{P}_i - 3.079)/(5.57 - \text{P}_i),$$

where P_i is the chemical shift of the inorganic phosphate peak relative to PCr peak. The pH_i of PC12 cells, thus calculated, was found to be 7.38. We could not calculate the pH_i in case of U-87MG cells as the PCr peak was not observed in these cells.

To determine the pathways of ATP production, the cells were perfused with the medium containing sodium azide and 2-deoxy-D-glucose (2-DG) during the NMR measurements. Sodium azide is an inhibitor of oxidative phosphorylation and prevents the production of ATP by blocking cytochrome oxidase, the last step in the electron transport chain. On the other hand, 2-DG blocks the glucose metabolism and inhibits the ATP production by glycolysis. On perfusing PC12 cells with the medium containing sodium azide, the PCr peak disappeared and a partial decrease in ATP was observed. However, on perfusing U-87MG cells with the medium containing sodium azide, a significant inhibition in ATP peaks was observed. Perfusion of PC12 cells with the medium containing 2-DG caused the appearance of 2-deoxy-D-glucose-6-phosphate (2-DG6P) with a complete inhibition of ATP peaks. U-87MG cells showed a small decrease in ATP signal on perfusing with the medium containing 2-DG, and an additional peak due to 2-DG6P was observed. These results are shown in Table 1 in

Table 1. The ratio of inorganic phosphate (P_i) and adenosine triphosphate ($\beta\text{-ATP}$) in PC12 and U-87MG cells perfused with HBSS

Perfusion medium	$\text{P}_i/\beta\text{-ATP}$	
	PC12 cells	U-87MG cells
HBSS + glucose	0.80 ± 0.15	0.33 ± 0.09
HBSS + glucose + NaN_3	0.97 ± 0.30	4.10 ± 0.54
HBSS + glucose + NaN_3 + 2DG	21.80 ± 1.83	4.27 ± 0.53

Cells were embedded in agarose gel threads and were perfused with HBSS during the NMR measurements. NaN_3 or NaN_3 + 2DG were added to the perfusate one hour before starting the experiments and were present during the data collection. $\text{P}_i/\beta\text{-ATP}$ ratio given is the mean \pm SE of three experiments.

terms of the ratio of P_i and $\beta\text{-ATP}$ ($\text{P}_i/\beta\text{-ATP}$). In presence of NaN_3 in the perfusion medium, no appreciable change in the ratio of $\text{P}_i/\beta\text{-ATP}$ was observed in PC12 cells while U-87MG cells showed an increase in $\text{P}_i/\beta\text{-ATP}$ ratio. On addition of 2-DG to the perfusion medium the $\text{P}_i/\beta\text{-ATP}$ ratio showed no change in U-87MG cells, however, in PC12 cells an increase in the same was observed. In PC12 cells, the inability of sodium azide to inhibit the ATP peaks indicates that glycolysis also contributes to ATP generation. This is further confirmed by inhibition of ATP peaks in the presence of 2-DG. Thus in PC12 cells, ATP is generated by both glycolysis and oxidative phosphorylation. On the other hand, in U-87MG cells, a significant inhibition of ATP by sodium azide and no further inhibition by 2-DG suggest that oxidative phosphorylation is the major pathway of ATP production.

The present study has shown that the cells of neuronal and glial origin have some differences in their energy metabolic pathways. These cells appear to differ in their energy metabolism and the differences in the cellular energy metabolism may probably be due to their specialized functional role.

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Anomalous helium emission: Precursor to earthquakes

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Helium content at the thermal springs at Bakreswar, near Calcutta, was observed to vary. These variations appear correlated to seismic perturbations. The position and distance of epicentre cannot yet be predicted from such correlation.

THERE is adequate evidence that the variations in the relative abundance of the constituents of terrestrial gas have certain degree of correlation with the perturbations within the mantle as well as the crust of earth¹. A recent report has indicated that there was a ten-fold increase in the radon content in bore hole gas prior to the earthquake at Kobe, Japan². Anomalous high helium concentrations have also been reportedly observed in well waters and soil gases preceding an earthquake^{3,4}.

It is well established by now that noble gas such as helium and radon interact weakly with matter; thus, it is expected that there will be pronounced variations in their relative abundance for such perturbations just mentioned, compared to their chemically reactive counterparts, such as nitrogen and methane. In the case of radon on account of chemical non-equilibrium and short recoil length, the host rocks incorporating the parent Ra-226 migrates rather large distances in water and in soil⁵. However, its relatively short decay time (3.82 days) inhibits any substantial concentration, so that very marginal radon content contribute above background value⁶. Over the last several years, we have been observing large fluctuation in helium concentration in the

natural gas emanating from thermal springs⁷. Three mechanisms have been suggested so far to account for the anomalies observed for the constituents of the soil gas in seismic-induced disturbances: (i) relative increase in heat flow that enhances gas concentrations near the surface^{7,8}, (ii) stress-induced pore collapse resulting in an upward flow of deep-seated gas⁹, (iii) stress-induced microfracturing leading to an increase in outgassing¹⁰. The collected sample of natural gas issuing from thermal springs was measured analytically by two separate techniques. A gas chromatograph operational at Bakreswar, the site of investigation (about 200 kms from Calcutta), of Variable Energy Cyclotron Centre was used to determine the relative abundances of helium and associated gases. Furthermore, the relative abundance of helium was determined by an absolute method based on the technique first developed by Frost¹¹.

We found the helium abundance at Bakreswar to be a variable quantity in general. The computed average of helium abundance, taken from recorded data of the diurnal readings for a five-year period, 1987-1991 was around 1.8%. We call this the characteristic 'helium index' for the spring. The normal value of the emanation rate during the quiescent state was close to the above figure with approximately $\pm 0.2\%$ variation. However it had been observed that the index began to fall very gradually two to three weeks prior to an impending seismic disturbance, known as earthquakes. The index tended to reach the minimum value four to five days before the triggering of the quake. This scenario was immediately followed by a sharp rise in the index, reaching its highest value two to three days prior to the occurrence of the quake, the peak value being two to three times the minimum value. Indeed, the helium content increased to 7.3% immediately prior to the volcanic eruption at Barren Island in July 1991 (ref. 12).

The measured magnitude of the helium concentration at Bakreswar for October in 1991 is plotted in Figure 1; corresponding data for January in 1992 is in Figure 2 and for the subsequent month of April 1992 is shown in Figure 3.

It was generally seen that the profiles had an irregular oscillatory pattern. This is expected since the helium contained in any underground environment is at best in a fragile state of equilibrium. It should also be mentioned that the presence of other diluting gases such as N₂ and CH₄ affected very feebly the outflow of helium as observed by Reimer⁴ in soil gas. For large scale deviations ($d > 3\sigma$) one has to thus look for catastrophic causes such as earthquakes as suggested by Scholz *et al.*¹³. In order to judge the long term and background behaviour of helium changes, emission graphs for the period June 1991 to October 1991, January 1992 to July 1992 and January 1993 to October 1993 (Figures 4-6) are given. The spring gas was collected

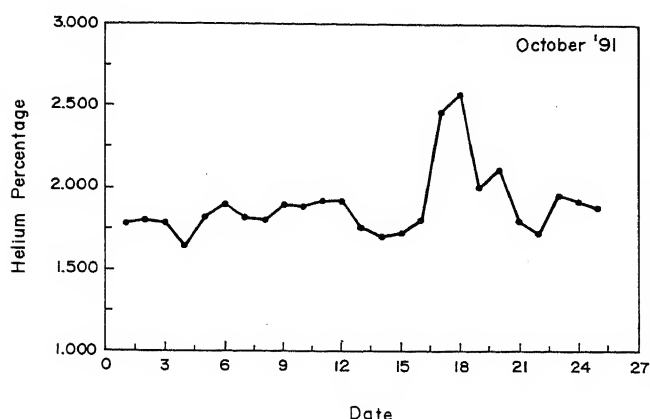


Figure 1. Variations of diurnal helium content during October 1991. The peak on 18 October precedes the Uttarkashi Earthquake on 20, 1991.

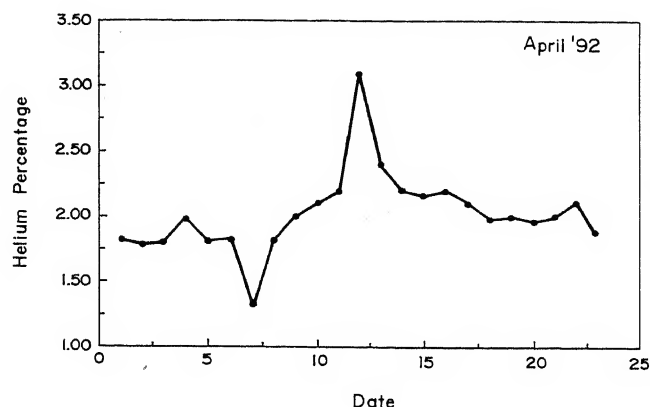


Figure 3. Helium variations for April 1992. The peak on 12 April corresponds to tremor at Shillong on 15 April 1992.

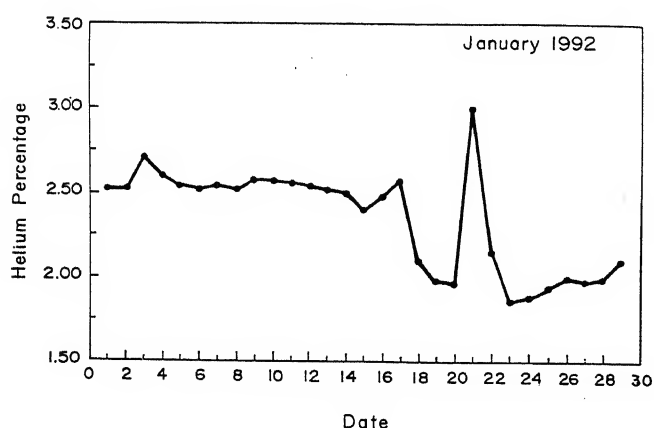


Figure 2. Helium variations for January 1992.

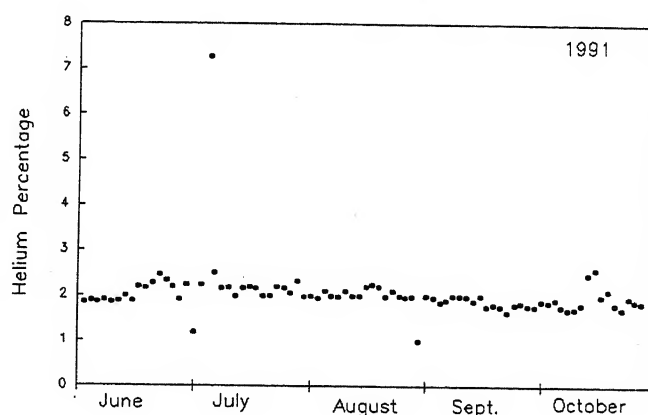


Figure 4. Temporal variations of helium in 1991 from June to October.

beneath a water column about 1.2 m. One can therefore preclude effects caused by meteorological conditions such as atmospheric pressure, wind speed, precipitation and temperature variations. As the springs lie in a relatively secluded area, influence of other non-tectonic factors such as mining, industrial activity, dam building or even water pumping is unlikely.

Our observations tend to indicate that the gas anomalies are reflected even at large distance from the centre of the quake. However, we could not correlate the observed increase in helium on January 21, 1992 to any specific tremor. Nonetheless, the peak may well be a manifestation of the reported seismic disturbances in Ionian sea; the Kamchatka peninsula and Japan's Honsu Island around January 23–25, 1992. It is also known that a change in strain associated with an earthquake by only 10^{-8} will lead to a change in the helium content¹⁴. Such gas anomalies and seismic disturbances are possible

incidental results of the same cause, the strain changes that take place within the earth's crust. Because of the large disparity in the magnitude of the energies involved, gas anomalies show up much earlier.

In some cases fluctuations as high as six to seven times the standard deviations have been noticed. It has not been possible so far, as mentioned, to predict with reasonable precision the distance of the epicentre of the quake or its location with relation to the thermal spring. One can only predict two or three days prior to the triggering of an earthquake somewhere! Work is in progress to estimate the radial distance of the epicentre from the spring site through multiple observation points. Although, as yet, there is no total one to one correspondence between earthquake and anomaly occurrences, we would venture to predict the following about the possible increase in the relative abundance of helium scenario just before the earthquake, admittedly rather

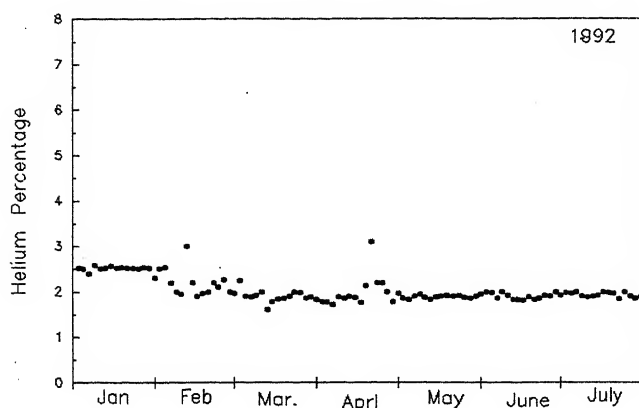


Figure 5. Temporal variations of helium in 1992 from January to July.

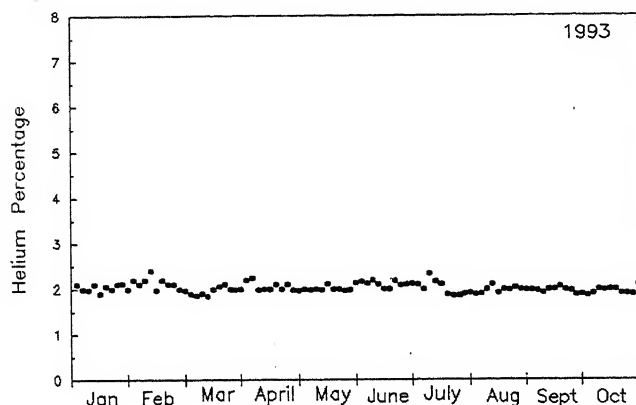


Figure 6. Temporal variations of helium in 1993 from January to October.

speculative, but interesting. Gradual accumulation of stress within the rock structure results in rise of pore pressure. The typical precursor of earthquakes, in general, is a steady rise in the pressure around the epicentre of the quake, at or around a yet unknown critical pressure of the system. Rather like more familiar phase transition of the system at a critical pressure essentially gives in with a sudden release of the pressure. With the rise of pressure the amplitude of the elastic shocks propagating around the centre increases as it approaches towards the triggering point. The shock waves, associated with the pressure release is the quake. With release of the pressure the helium stored in the rock matrix, aquifer or produced *in situ* tends to escape in larger volume as observed. The shocks travel a fairly large distance indeed, the very characteristic of helium is such that sources of helium turn out rather sensitive even at a very large distance. The speculative scenario, just prescribed has analogies with the bounce of supernova explosion or even head shocks or slide splash in nuclear physics^{15,16}; a geological analogue in large scale is yet to be worked out.

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Modelling of aeromagnetic anomaly and its implication on age of emplacement of ultramafic-mafic-alkaline complex at Jasra, Assam

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The aeromagnetic anomaly around Jasra in Assam, based on which the Atomic Minerals Division had discovered an ultramafic-mafic-alkaline carbonatite complex¹ has been modelled. Inferred direction of magnetization of the source body suggested the age of emplacement of the source to be around Jurassic period which is correlated with the time of breaking up of the Gondwanaland and the northward drift of the Indian plate and crustal upheavals.

THE aeromagnetic survey carried out by the National Geophysical Research Institute (NGRI), over an area of about 14000 km², covering parts of Garo hills, Shillong plateau, and Khasi hills region of Meghalaya and Assam

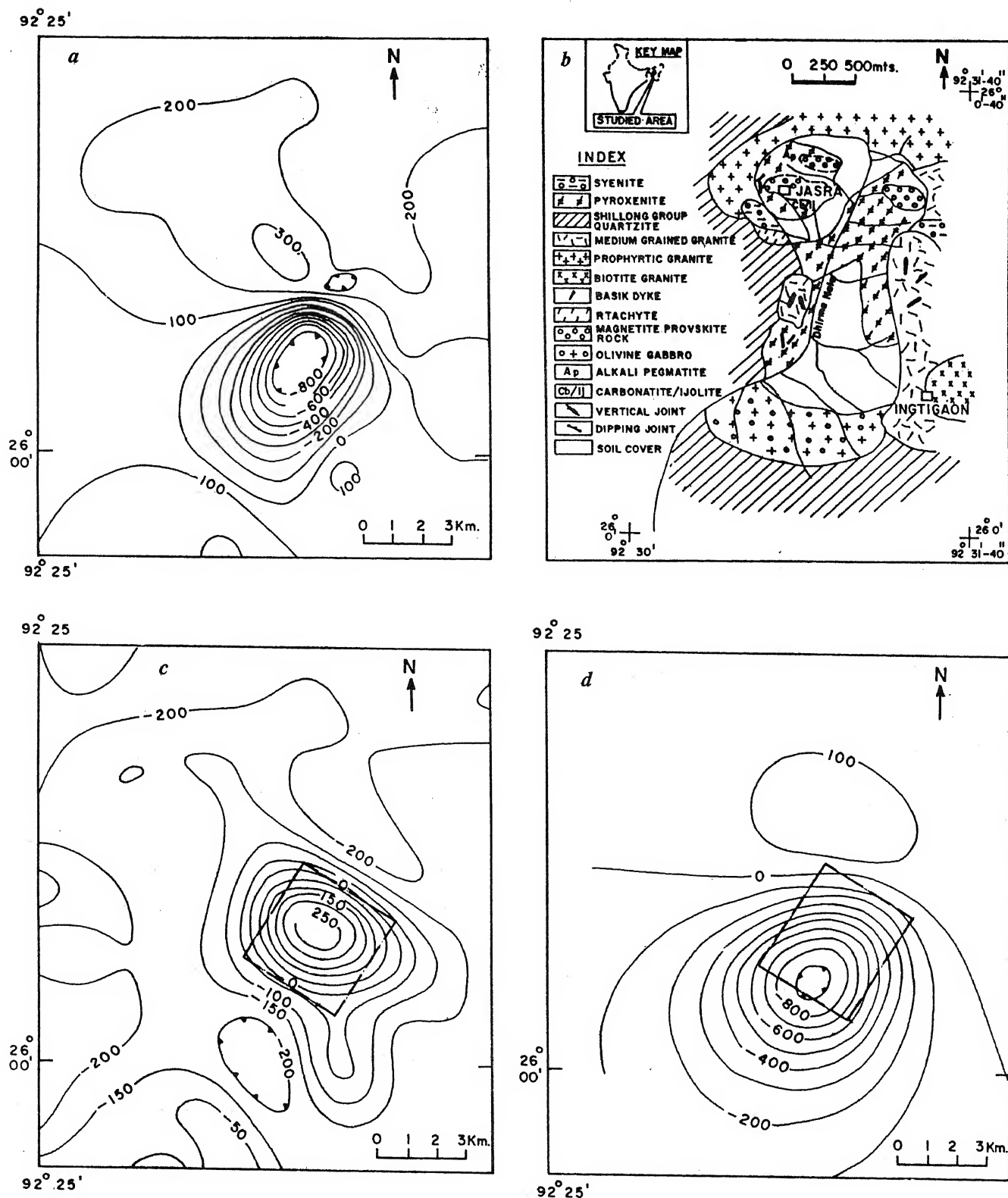


Figure 1a-d. a, Aeromagnetic anomaly around Jasra; b, geological map of Jasra area (after Mamallan *et al.*¹); c, the complex gradient map of Figure 1a showing the approximate boundaries of the source; and d, model anomaly of the interpreted parameters.

has brought out a number of important magnetic anomalies and structures. The recommendations suggesting important locations for mineral exploration were submitted as a technical report² to the North Eastern Council (NEC). One of the important aeromagnetic anomalies recommended for ground followup is the region around Jasra in Assam for the possible occurrence of ultramafic suite of carbonatite/pyroxenite rocks similar to those occurring in the Sung valley. The survey was carried out with a rubidium vapour magnetometer, designed and fabricated by the NGRI, and this region was flown on lines spaced at one kilometer apart at an altitude of 1380 m above sea level. The aeromagnetic anomaly around Jasra (after removing the IGRF) recommended for ground follow up is shown in Figure 1 a.

Based on the clues provided by aeromagnetic data and Landsat-TM imagery, the ground follow up work by the geologists of the Atomic Minerals Division (AMD) led to the discovery of an ultramafic alkaline (carbonatite) complex at Jasra, Assam and was described as a new find¹. This complex (Figure 1 b) consists dominantly of pyroxenites (with layers of titanomagnetite) with subordinate amounts of syenitic, alkali gabbroic bodies and very minor veins of ijolite, carbonatite and basic dikes. The pyroxenite body, together with other magnetizable minerals, produced an intense aeromagnetic anomaly described in the following.

The aeromagnetic anomaly associated with this complex shown in Figure 1 a, consists of a dominant low of about 800 nT amplitude with a small high on its northern side. The shape of the anomaly is not normal for this latitude for magnetization by induction and therefore suggests the presence of remanant magnetism in the source body. To infer the nature of the causative source and its magnetization, modelling of magnetic data has been done. The approximate outline of the source has been delineated from the 'complex gradient' map (Figure 1 c). The edges of the causative source appear as maxima on 'complex gradient' maps for wide and shallow sources; and for sources whose depth to top is roughly equal to the half-width, the half-maxima positions delineate the boundary of the source³. Modelling results indicated that the depth (1500 m below flight height) to the top of the source is roughly equal to the half-width of the source. Therefore, in the present case, the outline of the causative source is located at the

half-maxima positions of the complex gradient map and is marked on Figure 1 b. Interpretation of the north-south principal profile by using the 3-D vertical prism model provided the depth to the top, width and the magnetization of the source. The model anomaly of the interpreted parameters is shown in Figure 1 d. The depth to the top of the source was inferred to be about 70 m below the ground surface. The dimensions of the source obtained from the modelling are 2.5×3.5 km and appear to be larger than the outcropping complex. This may be due to widening of the source at depth. The intensity of magnetization is about 500 nT. The direction of magnetization (I) of the source is obtained as -63° . This parameter can be used to calculate the palaeolatitude (λ) by using the standard relation $\tan(I) = 2 \tan(\lambda)$. In the present case,

$$\tan(-63) = 2 \tan \lambda$$

$$\therefore \lambda = -45^\circ = 45^\circ \text{S}.$$

The palaeolatitude of -45° suggests that this part of the continent was located at 45° south latitude (i.e. in the southern hemisphere) at the time of emplacement of this intrusive source. The position of the north-eastern India at this latitude corresponds to the Jurassic period⁴ (125–175 my), which is in agreement with the reported ages⁵ of 156 ± 16 Ma and 149 ± 5 Ma for the alkaline magmatism in this region and is correlated with the time of breaking up of Gondwanaland and northward drift of the Indian plate and crustal upheavals.

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Further evidences of homeotic transformation in anuran tadpoles

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In this communication we report the development of ectopic supernumerary limbs (ESLs) from the cut ends of tails of *Bufo melanostictus* (Anura: Bufonidae), *Microhyla ornata* (Anura: Microhylidae) and *Hoplobatrachus tigerinus* (Anura: Ranidae) tadpoles exposed to various concentrations of vitamin A (Palmitate) solution. In addition, several other deformities (tail abnormality, outgrowth on tail tips, etc.) have also been recorded. Like the previous reports, the ESLs are all hindlimbs and comprise of both normal and abnormal hindlimb elements.

THE effect of tail tissue, its skin epidermis and dermis on the limb regeneration of salamanders *Triturus viridescens*¹ and *Siredon mexicanum*² had enough basis to influence researchers working on amphibian regeneration. Based on this, Iten and Bryant^{3,4} reported the stages of tail regeneration and regeneration from different levels along the tail of the newt, *Notophthalmus viridescens*. Preceding the above finding was the report by Niazi and Saxena⁵ highlighting the effect of vitamin A on tail regeneration of anuran tadpoles. Moreover, the reports on the inhibiting influence of vitamin A on tail regeneration in *Bufo andersonii*⁶, *Xenopus laevis*, *Notophthalmus viridescens* and *Ambystoma mexicanum*

tadpoles⁷ confirmed the above report. In contrast, the inhibiting influence of vitamin A was prevented by the use of sulphadiazine⁸ showing the antagonistic effects on vitamin A for tail regeneration of *B. melanostictus* tadpoles.

The finding demonstrating the development of ectopic supernumerary limbs (ESLs) at the site of tail amputation in *Uperodon systoma*⁹, was an ultimate breakthrough on vitamin A effect on the regenerating capacity of tails in anuran tadpoles. The phenomenon known as homeotic transformation was the first report in vertebrates, mediated through vitamin A. Thereafter, homeotic transformations have been reported in *Polypedates maculatus*¹⁰⁻¹⁴ (rhacophorid), *Tomopterna rolandae*^{13,15,16} (ranid), *Rana limnocharis*¹³ (ranid), and *Rana temporaria*¹⁷ (ranid) tadpoles exposed to various concentrations of vitamin A solution. Here we report additional information on homeotic transformation in three other anuran species.

The tadpoles of *B. melanostictus*, *M. ornata* and *H. tigerinus* used in the experiment were reared in the laboratory from egg clutches collected from breeding grounds during July 1994. Amputation was performed on tadpoles at Gosner stage 26 (hindlimb bud stage)¹⁸. The tadpoles were narcotized in 1:400 solution of MS222 in conditioned water. 128 of *B. melanostictus*, 60 of *M. ornata* and 9 of *H. tigerinus* tadpoles were used in the study. After tail amputation, the tadpoles were transferred to amphibian ringer and kept for about 10 min for recovery from anaesthetic condition and for

Table 1. Effect of vitamin A solution at the site of amputated tail of *Bufo melanostictus*, *Microhyla ornata* and *Hoplobatrachus tigerinus*

Species	Concentration of vitamin A	Hours of exposure	Number of tadpoles	Abnormal tail	Normal tail	Survival time range (days)	Number of tadpoles with ESLs
<i>B. melanostictus</i>	10 IU/ml vitamin A	24	5	2	3	5-96	—
		48	13	9	4	7-75	—
		72	27	20	7	6-46	—
		96	16	15	1	13-48	2
		120	13	9	4	7-50	1
		144	10	9	1	6-50	—
	15 IU/ml vitamin A	24	13	8	5	8-89	—
		48	11	9	2	9-70	—
		72	12	10	2	5-44	1
		96	8	5	3	5-44	—
	Control	—	5	—	5	15*	—
<i>M. ornata</i>	30 IU/ml vitamin A	72	55	19	36	6-86	1
	40 IU/ml vitamin A	48	5	2	3	3-86	1
	Control	—	5	—	5	40*	—
<i>H. tigerinus</i>	20 IU/ml vitamin A	96	9	6	3	20-25	1
	Control	—	5	0	5	25*	—

*Days for completion of metamorphosis; ESLs, Ectopic supernumerary limbs.

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coagulation of blood at the amputation site. For each species, the control group consisted of 5 tadpoles, reared in 500 ml of conditioned water after tail amputation. The rest of the tail amputated tadpoles were treated with various concentrations of vitamin A solution for different time intervals. After exposure to vitamin A solution, the tadpoles were reared in conditioned water

until death or emergence of forelimbs. Table 1 shows the number of tadpoles, concentration of vitamin A solution and hours of exposure.

In *B. melanostictus* (Figure 1 a-f), the control tadpoles metamorphosed within 15 days of amputation and there was no structural abnormality in both the fore- and hindlimbs. Some of the experimental tadpoles survived up to a maximum of 96 days in 24 h exposure to vitamin A solution. However, none of the tadpoles metamorphosed and they died at some stage of development, during the period when a tail stump differentiated into a hindlimb. Out of 128 experimental tadpoles (Table 1), 25% regenerated normal tail. The rest developed abnormal tails with various kinds of morphological abnormalities. The regenerated tails had tail fins that did not cover the tail tip and there was cellular mass protruding from the tip of the axial tissue. In some, the dorsal fin extended along with the tail musculature curving downwards, while the ventral fin narrowed and was suppressed from where the tail tissue began its curve. Others had bent tail fins, ventral fin broader and covering the tail tip, while the dorsal fin narrowing mid way. A swelling that developed in the tadpoles at the amputation site had reduced tail fins. In addition to the abnormal tail tips, the fore- and hindlimbs were also deformed. The hindlimbs had reduced shank and the forelimbs were without distinct humerus, radio-ulna and with 6 fingers. There was also the development of extra paired hindlimbs at the site of the growth of normal hindlimbs.

Ectopic development of limbs at the amputation site was observed in 2.3% of experimental tadpoles. In addition, there was also regeneration of tail with laterally curved axial tissue and tail fins along with the development of ESLs. The ectopic hindlimbs also developed in pairs having distinct thigh, shank, ankle and digits. In some, the shank and ankle were suppressed, thighs fused and the 4th and 5th digits joined (Table 2).

In *M. ornata* (Figure 2 a-d), the control tadpoles metamorphosed within 40 days of amputation, without exhibiting any abnormality. The experimental tadpoles (Table 1) had a maximum survival period of 86 days and 65% of them developed normal tails while the rest regenerated abnormal tails. The abnormalities included

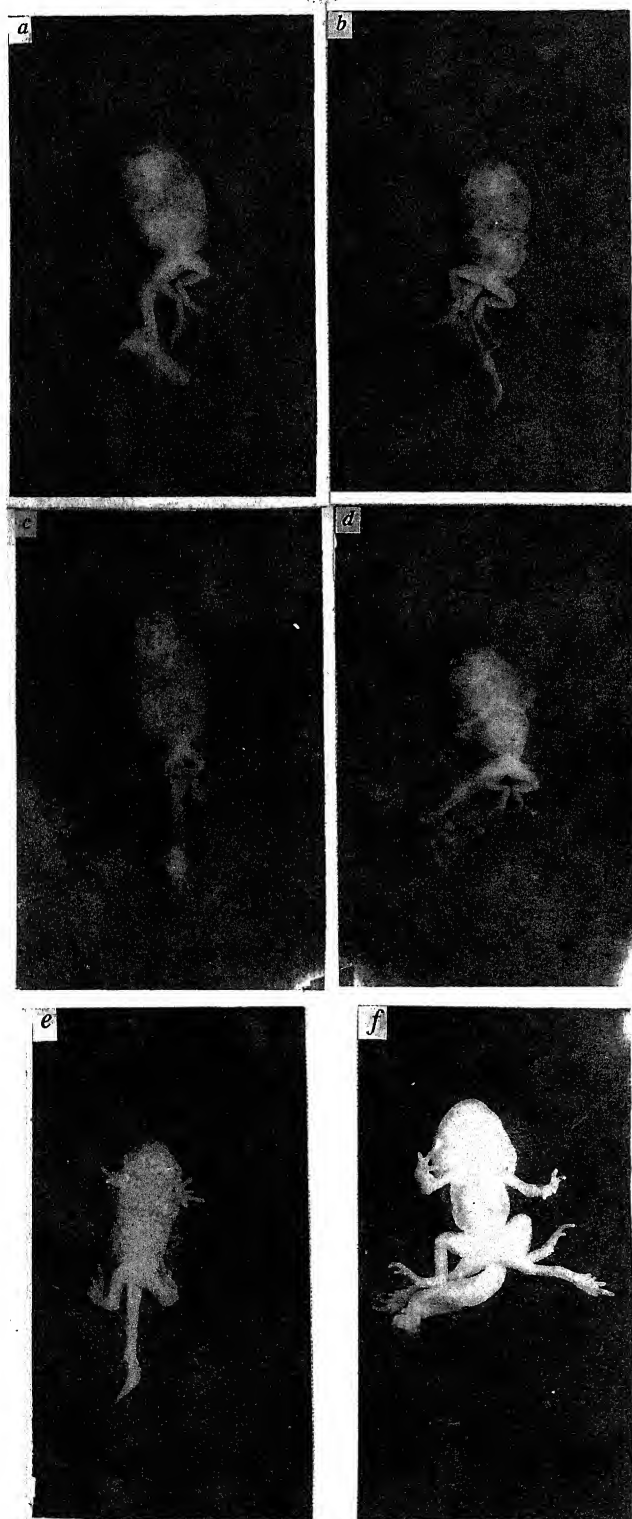
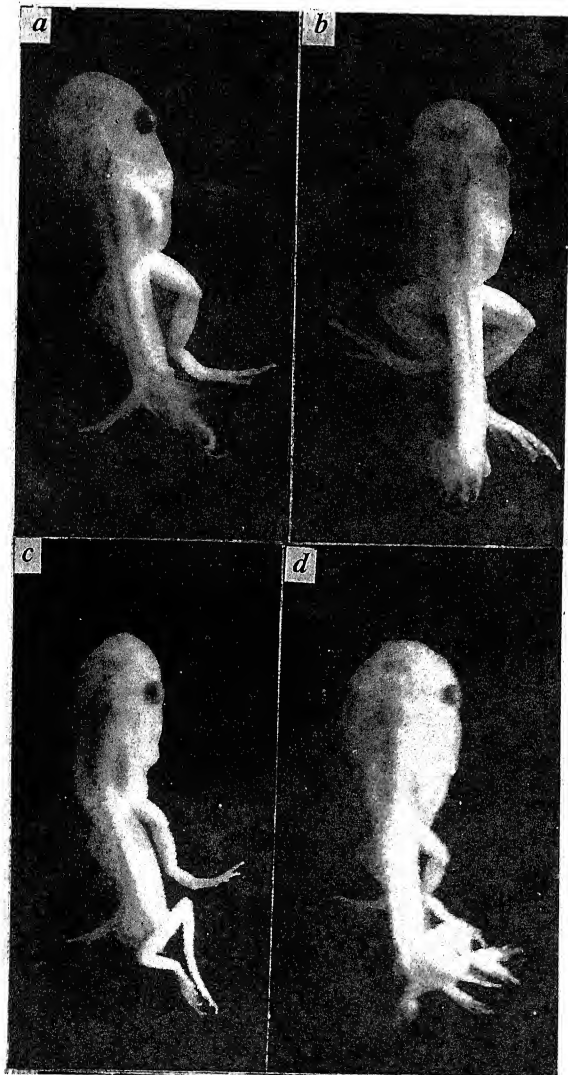


Figure 1 a-f. *Bufo melanostictus*. a, Development of cellular outgrowth and a pair of abnormal ESL with suppressed shank and ankle (exposure: 10 IU vitamin A for 120 h); b, Development of three normal ESLs (exposure: 10 IU vitamin A for 96 h); c, Development of a pair of ESL at the limb bud stage, along with a swelling (exposure: 10 IU vitamin A for 96 h); d, Development of a pair of abnormal ESL with jointed thighs, 4th and 5th digits of one ESL fused (exposure: 15 IU vitamin A for 72 h); e, Development of abnormal tail tip, the hindlimbs with reduced shank and the forelimbs without distinct humerus, radio-ulna and with six fingers (exposure: 10 IU vitamin A for 96 h); f, Development of abnormal tail tip and extra pair of hindlimb at the site of the growth of normal hindlimbs (exposure: 10 IU vitamin A for 96 h).

Table 2. Analysis of ESLs of *Bufo melanostictus*, *Microhyla ornata* and *Hoplobatrachus tigerinus*

Species	Group	Number of tadpoles with ESLs	Number of ESLs	Normal ESLs	Abnormal ESLs	Abnormality/normality of the ESLs
<i>B. melanostictus</i>	10 IU (96 h)	2	(i) 1 (ii) 3	— 3	1 —	Limb bud, thigh, shank ankle and digits, well developed
	10 IU (120 h)	1	2	—	2	Shank and ankle, suppressed in both the ESLs
	15 IU (72 h)	1	2	—	2	Jointed thighs digits 4th and 5th attached
<i>M. ornata</i>	30 IU (72 h)	1	4	2	2	Jointed thighs
	40 IU (48 h)	1	2	2	—	Distinct thigh shank, ankle and digits
<i>H. tigerinus</i>	20 IU (96 h)	1	2	—	—	Limb bud



growth of irregular cell mass at the amputation site, deshaped tail fins, loss of fins and small outgrowths ventro-laterally on the regenerating tail, and development of ESLs (3.3%) at the site of amputation. The ESLs besides having distinct thigh, shank, ankle and digits also had fused thigh (Table 2).

In *H. tigerinus* (Figure 3 a-f), the control tadpoles metamorphosed within 25 days of amputation and like the other two species, there was no abnormality in their limb or tail. Like the controls some of the experimental tadpoles survived for a maximum of 25 days, but none of them metamorphosed (Table 1). The abnormalities in tail regeneration included the development of irregular cell mass at the site of amputation, deshaped tail musculature and fins and development of ESLs. The abnormality was 66.7% and out of the total abnormal tadpoles, 11.1% showed ectopic limb development covered externally by the swellings (Table 2).

The above results indicate variation in the time period of completion of metamorphosis in the control groups and this is species-specific. In the experimental tadpoles, the maximum survival time was also recorded for *B. melanostictus* exposed to 10 IU/ml vitamin A solution for 24 h. Besides, the highest percentage (75%) of abnormality was also recorded for the species. The number of ESLs was the highest (4) in *M. ornata*. All the tadpoles of *H. tigerinus* sustained a high dose of vitamin A (20 IU) solution for a longer period and mortality was recorded from the 20th day of treatment.

Figure 2 a-d. *Microhyla ornata*. a, Regeneration of a curved tail and deshaped tail fin (exposure: 30 IU vitamin A for 72 h); b, Cellular outgrowths at the amputation site (exposure: 30 IU vitamin A for 72 h); c, Development of a pair of ESLs (exposure: 40 IU vitamin A for 48 h); d, Development of 4 ESLs; one pair normal and the other pair abnormal with jointed thighs (exposure: 30 IU vitamin A for 72 h).

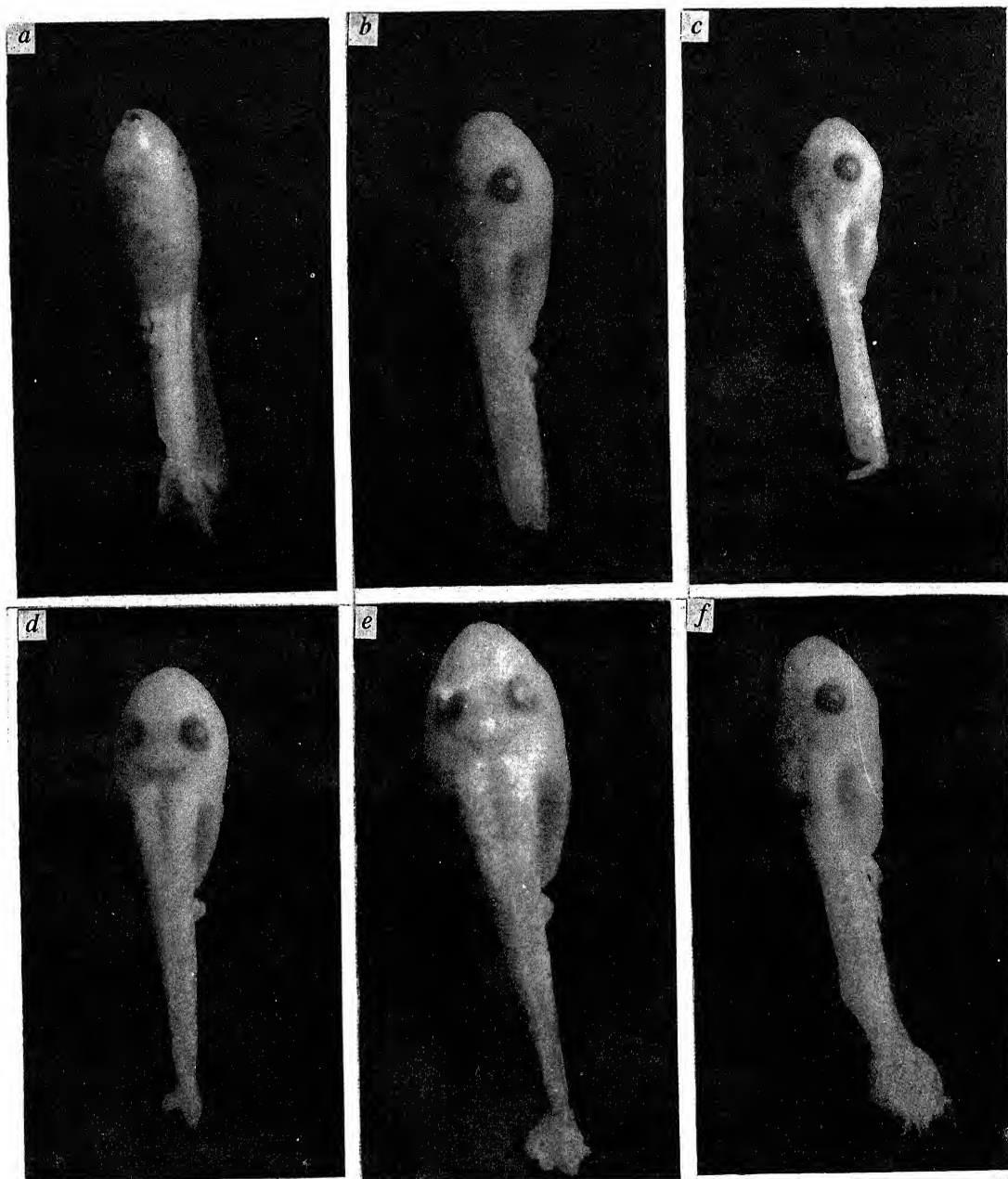


Figure 3 a-f. *Hoplobatrachus tigerinus* tail amputated tadpoles, exposed to 20 IU vitamin A solution for 96 h. *a*, Development of a cell mass and folding of the tail fins at the amputation site; *b*, A small swelling at the amputation site covered by the tail fins; *c*, Loss of fins at the tail tip; *d*, Abnormal tail regeneration, with a small outgrowth at the tail tip; *e*, Development of irregular cell mass at the tail tip; *f*, Development of irregular cell mass with ESLs (minute).

The experimental tadpoles of all three species exhibited delayed growth as reported earlier for *R. temporaria*¹⁷ (ranid) and *P. maculatus*¹⁴ (rhacophorid) tadpoles. In addition, the mortality was not time-dependent and there was no correlation between mortality and the duration of exposure to vitamin A solution, unlike that reported for *P. maculatus*¹⁴, *B. andersonii*^{5,6} and *R. cyanophlyctis*^{6,19}.

The experimental tadpoles also regenerated abnormal

tails as reported for *U. systoma*⁹, *B. andersonii*^{5,6}, *X. laevis*⁷, *N. viridescens*⁷, *A. mexicanum*⁷, *P. maculatus*^{12,14} and *T. rolandae*¹⁵. The most fascinating finding was the development of ectopic hindlimbs in all the species. However, the percentage (4.1%) was lower than earlier reports^{12,14,17}. Vitamin A palmitate administered for at least 48 h showed the induction of limbs at the site of tail amputation in *B. melanostictus*, and *M. ornata* tadpoles. *H. tigerinus* tadpoles needed longer exposure

(72 h) to vitamin A solution. Hence, it is concluded that 48–72 h was the required duration of treatment for the development of ectopic limbs as has also been reported for *R. temporaria*¹⁷ (3 days as the threshold level) and for *P. maculatus*¹⁴ (48 h as the minimum exposure time). However, in *P. maculatus*¹², *T. rolandae*¹⁵ and *R. limnocharis*¹³ ectopic limbs developed when the exposure time was for 24 h. Thus, it can be inferred that the induction of ectopic limbs is not time-dependent as otherwise proposed by Maden¹⁷.

It has been interpreted that hindlimb development is marked by the rising levels of thyroid hormone (TH)²⁰. It is likely that during the regeneration process²¹ at the amputated site, the TH combines with vitamin A palmitate and alters the tail hox code while repressing other genes resulting in the formation of limb blastema cells, particularly the ones that give rise to hindlimb fields¹⁷.

The effect of vitamin A palmitate at the tail amputated site of tadpoles causing homeotic transformation may be attributed to the conversion of the tail blastema cells to that of limbs. This changes the positional values at the tail tip. Subsequently, rearing in conditioned water after exposure to vitamin A, reverses the polarity along the rostral–caudal axis to generate ectopic limbs²². Some of the ectopic limbs develop from cellular mass at the site of amputation and mostly in a ventro-lateral direction similar to those observed in *P. maculatus*^{12,14} and *T. rolandae*^{13,15}. This is comparable to the finding in *B. andersonii*⁶ in which continued exposure to vitamin A palmitate resulted in the folding of the fin to form a pouch at the tip of the regenerating tail. The cellular mass which is formed prior to the development of ectopic limbs may act as a reservoir for the transformed cells which needs to be investigated through histology. There was also regeneration of a complete tail that followed the growth of ESLs in *B. melanostictus* tadpoles as reported in *P. maculatus*¹², *T. rolandae*¹⁵ and *R. limnocharis*¹³. This is expected, in accordance with the interpretation schematically proposed by Bryant and Gardiner²². However, the interpretation of development of paired ectopic hindlimbs²² is contradictory to the present finding as odd number (3) of ectopic limbs has been observed in *B. melanostictus* tadpoles as was also found in *T. rolandae*¹⁵ and *R. limnocharis*¹³. Further, with the growth of an abnormally regenerated tail, there was also the development of extra-paired hindlimbs at the site from where only a single pair of hindlimb was supposed to develop. This observation was previously reported in *B. vulgaris*²³ and again in *U. systoma*⁹. These deformities could be related to the embryonal malformations caused by vitamin A²⁴. In addition to the findings on *U. systoma*⁹, *R. temporaria*¹⁷, *T. rolandae*¹⁵, *R. limnocharis*¹³ and *P. maculatus*^{10,12,14}, the present study adds further to our knowledge on homeotic transformation in anuran tadpoles.

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How does the nocturnal animal, *Mus booduga*, programme its activity in response to varying durations of light and darkness?

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In the temperate zone, animals adjust to various durations of light and dark by scaling the duration of activity with the durations of light (diurnal animals) or dark (nocturnal animals). They are also capable of advancing or delaying their activity depending on the durations of light or darkness. This study examines whether tropical animals which do not normally experience much variations in durations of light and

darkness can also do the same. Experiments with the field mouse *Mus booduga* show that their behaviour with respect to scaling the durations of activity matches that of temperate animals but not so with respect to anticipating the onset of darkness. It is speculated that scaling of durations of activity (α) may not require prior experience while anticipating the onset of light or darkness (i.e. showing the appropriate phase angle difference) being a more complicated adjustment cannot be achieved without prior experience.

A fascinating aspect of animal behaviour is the impressive synchronization (entrainment) of activity/rest cycles to natural photoperiods. Thus, diurnal animals confine their activity period to the light phase (L) and rest period to the dark phase (D), while nocturnal animals do the reverse¹. This synchronization is maintained even under varying durations of light and dark in the laboratory, which may sometimes deviate considerably from the natural L:D ratios². But how do varying durations of L and D affect the durations of activity (referred to as α)? There is evidence³, for example, that chaffinches correspondingly increased their α as the duration of L was increased from 8 to 20 h. Even when wider ranges of durations of L (or D for nocturnal animals) are used (or are present under natural conditions such as in some parts of the world, for example) α shows an S-shaped curve. This means that α remains almost the same up to certain durations of light, then increases linearly with increase in light and beyond a certain point reaches a plateau⁴.

A related question is how varying durations of L and D alter the time difference (positive or negative) between the onset of L (or D for nocturnal animals) and the onset of activity. Such time difference is referred to as the phase angle difference (ψ) and may be measured as the difference between the onset of activity and onset of L (or D) (ψ_o), midpoint of activity and midpoint of L (or D) (ψ_m) or end of activity and end of L (or D) (ψ_e). Observations under naturally varying photoperiods have shown that under long day length conditions for example, diurnal animals start their activity late (i.e. have a negative ψ_o), reach the mid-point of their activity before the mid-point of L is reached (i.e. have a positive ψ_m) and reach the end of their activity a little before the end of L (i.e. have a positive ψ_e). Diurnal animals show exactly the opposite behaviour under short day conditions and nocturnal animals show similar behaviour with respect to long and short nights respectively^{4,5}.

While these phenomena (both scaling of α and phase angle difference with varying durations of L and D) have been investigated in the temperate zone, we know almost nothing about tropical animals, especially tropical mammals. Is it possible that since natural photoperiods are more or less constant in the tropics, these animals

are unable to respond in the manner described above when L:D ratios are altered drastically in the laboratory? This question motivated the present study.

Adult males of the nocturnal tropical field mouse *Mus booduga* ($n=6$) were obtained from fields near Madurai University campus (9°58'N, 78°10'E). They were maintained with *ad libitum* food and water in laboratory cages fitted with running wheels where an eccentrically placed magnet facilitated recording of locomotor activity. The rotations of the wheels were picked up by an Esterline Angus event recorder. The light intensity at the cage level during L phase was about 45 lx while absolute darkness prevailed during the D phase. The different LD duration ratios were achieved by altering the relative durations of L and D (by either lengthening a single light time (L) or a single dark time (D) as the case may be) while keeping the sum at 24 h. The various L/D ratios administered were: 22:2 h; 20:4 h;

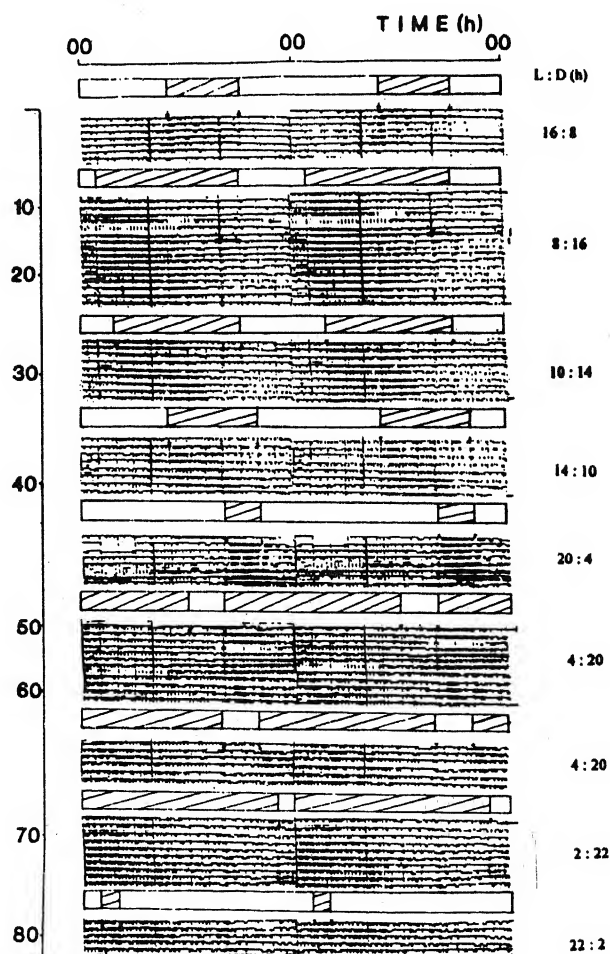


Figure 1. Actogram showing the activity pattern of an animal subjected to differing LD ratios. Open bars indicate durations of light time and filled bars indicate durations of dark time. The intensity of light during light phase was about 45 lx and absolute darkness prevailed during dark phase.

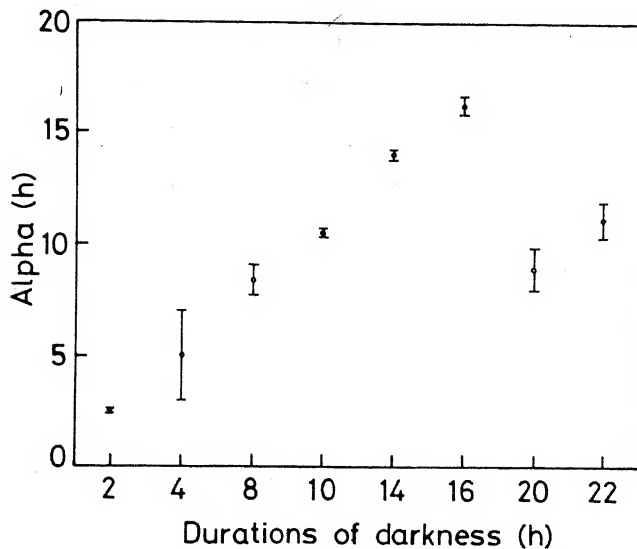


Figure 2. Durations of activity (α) as a function of durations of darkness. Mean \pm SD values are shown.

16 : 8 h; 14 : 10 h; 10 : 14 h; 8 : 16 h; 4 : 20 h and 2 : 22 h. Only the duration of light or dark was altered and the intensity of light during L phase was kept constant. Actograms were double plotted using conventional methods (Figure 1).

This study yielded two major results. One was that α did vary with durations of D (as expected in nocturnal animal) all the way from 2 to 16 h, although the positive correlation between α and D broke down after 16 h (Figure 2). This means that these tropical nocturnal animals were able to scale their α with varying durations of D much as temperate animals are capable of doing so, although they would not be expected to have adaptations to such drastic variations in LD ratios. It is true however, that the scaling of α with durations of D broke down when the duration of D was 20 or 22 h. Here α was 8.92 ± 0.96 and 11.13 ± 0.78 respectively. This breakdown of the scaling of α at extreme D durations suggests that the scaling observed when D was varied from 2 to 16 h is in fact an intrinsic property of the entrainment mechanism rather than a simple masking effect of light. In other words, the activity of this nocturnal animal was not merely suppressed by the presence of light. If that were so, there is no reason why α should not have kept pace with D even beyond 16 h. It can also be seen from Figure 1 that the scaling of α with durations of D from 2 to 16 h was quite independent of the phase in which the animal was when the LD ratios were varied. This again argues that the observed scaling of α with durations of D was not a masking effect as masking has shown to be phase dependent^{6,7}.

The second result of this study was that the phase angle differences did not correspond to the general

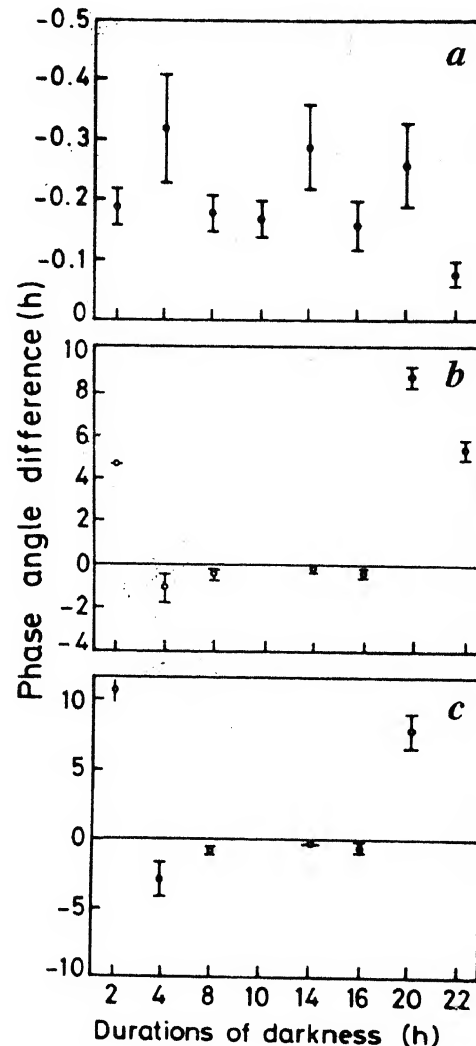


Figure 3. Phase angle differences as a function of durations of darkness. a, onset (ψ_0); b, mid point (ψ_m); c, end (ψ_e). Mean \pm SD values are shown. Data not available for 10 h of durations of darkness.

pattern observed in temperate zone animals described earlier. Indeed, there was no discernible pattern in the phase angle difference whether measured as ψ_0 , ψ_m or ψ_e , with increasing durations of darkness (Figure 3). The absence of differences in phase angle in response to varying LD ratios as expected in a temperate animal is not really surprising for *Mus booduga*. Tropical animals are not expected to be exposed to much drastic variations in LD ratios and are thus not expected to have the required adaptations. The longest night in Madurai for example is 12.5 h while the shortest night is 11.4 h. Studies on the tropical bat *Hipposideros speoris* have shown that these bats do seem to anticipate the onset of darkness⁸. However, since only the natural seasonal variations in LD ratios were used in this case, the bats may be expected to be able to be adapted to such variations.

The contrasting results with respect to the observed scaling of α with durations of D and the absence of

the expected patterns of differences in phase angles with varying LD ratios in the present study, raise an interesting question. How is it that a tropical animal, not used to much variations in LD ratios (in its recent evolutionary past) can still show the same behaviour as temperate animals with respect to scaling of α but cannot do so with respect to varying the phase angles. It is interesting to speculate that scaling of α with durations of D is a simpler phenomenon not really requiring prior experience or adaptation while varying the phase angles as temperate animals do is a more complicated phenomenon which involves anticipating the onset of light or darkness. This may be dependent on prior experience or adaptation. However, this speculation is based on this single result with a tropical animal. Given the extreme paucity of information on the behaviour of tropical animals under varying LD ratios and the unusual results obtained in this study, more work along these lines on other tropical animals, diurnal and nocturnal, is sorely needed.

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Potent amoebicides from plant extracts – An *in vitro* assessment with the gum-oleo-resin of *Commiphora wightii*

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The medicinal value of *Commiphora wightii* has been believed by tribals to be mainly due to its yield of guggulipid, which has been scientifically shown to have hypcholesteromic, anti-septic, anti-pathogenic, anti-parasitic properties. It is also used for non-specific diarrhoea and dysentery. Amoebic dysentery is a common disorder of a large number of people

in the tropics. In our studies we have reviewed the active principles of most known anti-amoebic plants. Further, we have tested the crude extracts of oleo-gum-resin obtained from *C. wightii* against *E. histolytica* NIH 200 using microdilution technique. They were found to be comparable with quassinoids; Ailanthinone and Bruceantin. The need for linkages between chemical characterization with established *in vitro* techniques is demonstrated.

AMOEBIASIS by *Entamoeba histolytica* is an important cause of dysentery. Recent global estimates indicate the increasing trend from 480 million people (excluding China) annually suffering from amoebiasis¹. The search for herbs and medicines for this scourge, from all possible sources is an ongoing exercise. Many natural products of plant origin are an important source of biologically active compounds and have potential for the development of novel antiprotozoal drugs as studied by *in vivo* and *in vitro* experimentations². Table 1 shows known anti-amoebic plants studied since the last decade for their active fractions and principles.

Commiphora wightii (Arnott) Bhand, (Burseraceae) is a small tree or large-sized shrub which produces a gum 'guggal' believed to have high medicinal value. It is commonly known as 'Indian bdellium' or 'guggal' in India³. It is found in the arid rocky tracts of Rajasthan, Gujarat, Karnataka and Maharashtra states of India; Sindh and Baluchistan states of Pakistan; Bangladesh and Arabia⁴. The trunk is knotty, outer bark comes off in rough flakes leaving an inner layer which is bright, shining and peels off in thin rolls like paper. The latex oozes out through wounds or cuts as a yellow fluid which hardens to form a golden brown, yellow or reddish brown oleo-gum-resin. Guggal gum is a mixture of 61% resin, 29.3% water, 0.6% volatile oil and 3.2% foreign matter³. Guggal gum is known for its therapeutic properties in various ailments, particularly arthritis, many vascular and neurological complications, hypercholesteremic conditions⁵, rheumatism and possesses anti-inflammatory activity⁶, in cure of ischaemic heart disease, obesity, neurological disorders, ills of syphylitic nature⁷, scrofulous infections, urinary disorders and a few skin diseases⁴. Its essential oil also possess antibacterial, antifungal and antihelminthic activity.

As one of the ingredients in 'Arogya Vardhini Bati', an Ayurvedic drug, *Commiphora* is used for the treatments of diarrhoea and dysentery in man and its efficacy has been tested both *in vitro* and *in vivo*⁸. Alcohol extract of its oleo-gum-resin was tested *in vitro* against axenic cultures of *E. histolytica* NIH 200 but proved less effective than *Curcuma zedoaria*⁹. The most optimal comparisons of other such plants which were similarly studied for their active principles are *Brucea javanica*, *B. antidysenterica* and *Simarouba amara* with quassinoids; Bruceantin and Ailanthinone as their active compounds.

Table 1. Different plants possessing antiamoebic potential along with their active fractions

Plant sps.	Family	Part used	Fraction/extract	Remarks and reference no.
<i>Allium sativum</i>	Liliaceae	Cloves	Crushed cloves	Allicin inhibits the growth of axenically cultured <i>E. histolytica</i> at 30 µg/ml provided cysteine was not present in the medium. The activity of gossypol, a polyphenolic compound was 11 and 39 times greater than those of metronidazole and emetine respectively ² .
<i>Gossypium herbaceum</i>	Malvaceae	Seed	Oil	
<i>Brucea javanica</i>	Simaroubaceae	Fruit	Petroleum ether fraction, aqueous fraction, chloroform fraction	A little activity was present in petroleum ether fraction of either species but chloroform fraction of the two was highly active ¹² .
<i>Simarouba amara</i>	Simaroubaceae	Stem	Chloroform fraction	
<i>Brucea antidysenterica</i>	Simaroubaceae	Fruit	Chloroform fraction	Rates of amoeba killings by metronidazole and bruceantin, a quassinoid, were similar at 16 to 30 fold lower water conc. of the latter ¹³ .
<i>Strychnos gossweileri</i>	Loganiaceae	Root bark	Alcoholic extract	Diploceline, a quaternary alkaloid isolated is active at 50 µg/ml on <i>E. histolytica</i> ¹⁴ .
<i>Euphorbia hirta</i>	Euphorbiaceae	Whole plant	Ethylacetate fraction	Quercitrin, a flavonoid was active as antidiarrhoeic from doses of 25 mg/kg onwards ¹⁵ .
<i>Alstonia angustifolia</i>	Apocynaceae	Root	Methanol extract	Macrocarpamine, an alkaloid was found to be the most potential antiamoebic compound but 4 times less potent than the standard drug, emetine ¹⁶ .
<i>Triclisia</i> sps.	Menispermaceae	Wood	Alcoholic extract	Armoline, isotrilobine and insularine were the most active bisbenzylisoquinoline alkaloids having IC ₅₀ in the range of 5–11 µM (ref. 17).
<i>Holarrhena antidysenterica</i>	Apocynaceae	Fruit	Alcoholic extract	Conamine, holarrhimine, conkurchine, etc. were the most active alkaloids ¹⁸ .
<i>Strychnos</i> sps.	Loganiaceae	Stem	Alcoholic fraction	Usambarine and usambarensine were the most selective alkaloids. The latter possesses activities similar to those of emetine and metronidazole ¹⁹ .

In the present study an attempt has been made to test the *in vitro* anti-amoebic potential of various crude extracts of *C. wightii* using the microdilution technique. Gum resin was collected from Ajmer district (Rajasthan) and air-dried. Gum-resin (40 g) was taken, ground and extracted with 300 ml of ethyl alcohol for 10 days at 60–80°C using soxhlet apparatus. Ethanol containing the compounds was filtered and the filtrate heated at a relatively low temperature to evaporate ethyl alcohol. The ethanol soluble portion contributes about 75% of the gum resin (28.1 g). This was followed by cold extraction using petroleum ether (60–80°C). About 150 ml of petroleum ether was added to the left compound and kept for 2 days without heating so as to enable the oils and other compounds to solubilize in it. This was heated for about half-an-hour at 30°C, filtered and the filtrate was heated up to 30°C till petroleum ether evaporated. The residue was extracted with about 200 ml of chloroform and heated at 40–50°C for half-an-hour. It was cooled down, filtered and concentrated after heating. The leftover gum in the extractor, from which compounds were extracted initially using ethanol, was taken out in a conical flask, water was added to it and kept overnight. Next day it was filtered and the filtrate was heated to evaporate water. Thus, the three extracts, viz. 9.2 g of petroleum ether (yield 32.7%, w/w), 15.3 g of chloroform (yield 54.4%, w/w) and 3.6 g of aqueous (yield 12.7%, w/w) were ready for testing. *In vitro* testing against *E. histolytica* was carried out thrice in

triplicate cultures using microdilution technique. Ethanol (50 µl) was added to 10 mg of each extract followed by 950 µl of fresh culture medium. They were further diluted twice with culture medium so as to obtain a concentration of 0.1 mg/ml. These were considered as stock solutions and from them 6 serial dilutions were prepared, as shown in Table 2, using fresh culture medium (final volume, 170 µl).

Each microtitre plate included metronidazole as a standard amoebicidal drug, control wells (culture medium plus amoebae), test wells (culture medium plus crude extracts) and a blank (culture medium only). Amoebae cell suspension 170 µl was added to the test and control wells in the plates so that the wells were completely filled (total volume 340 µl). Plates were sealed with expanded polystyrene, secured with tape and placed in an incubator at 37°C. After 48 h of incubation, inhibition of growth was assessed by measuring OD with an ELISA reader at 540 nm. When compared against the standard drug metronidazole, the chloroform extract was found twice as promising as metronidazole in its activity (Figure 1). The high content (8.24%) of chloroform extract which was taken from the original 40 g of the gum resin, and also its comparably higher activity, together makes the chloroform extract an important target for further characterization. In a separate study done, the chloroform extract of *C. wightii* was shown to contain guggulsterol I–V, guggulsterones E and guggulsterones Z¹⁹. Table 2 shows comparable effectiveness

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Table 2. Relative effectiveness of various extracts and metronidazole (standard drug)

Conc. of extract/drug (µg/ml)	Log of conc.	Inhibition by metronidazole extract (%)	Inhibition by chloroform extract (%)	Inhibition by aqueous extract (%)	Inhibition by petroleum ether extract (%)
0.125	-0.9030	23.86	43.79	43.09	31.80
0.25	-0.6020	38.59	52.19	51.01	38.48
0.5	-0.3010	53.33	60.59	58.94	45.16
1	0	68.07	68.98	66.86	51.84
2	0.3010	82.81	77.38	74.79	58.52
4	0.6020	97.54	85.78	82.71	65.20

IC₅₀ for the standard drug, metronidazole = 0.43 µg/ml.

IC₅₀ for chloroform extract = 0.22 µg/ml.

IC₅₀ for aqueous extract = 0.24 µg/ml.

IC₅₀ for petroleum ether extract = 0.88 µg/ml.

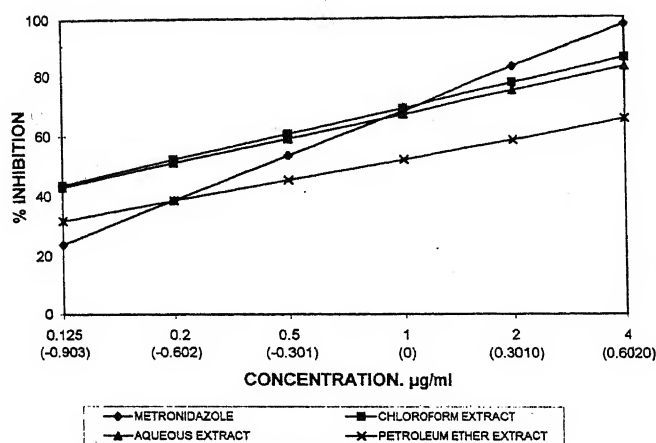


Figure 1. Activity of standard drug metronidazole, chloroform extract, petroleum ether extract and aqueous extract obtained from gum-oleo-resin of *Commiphora wightii* against *Entamoeba histolytica* (NIH 200) *in vitro*. (Log of the corresponding X-axis values are given within the brackets).

of three crude extracts of *C. wightii* and the standard drug metronidazole.

The active principles from *C. wightii* have been classified into four fractions, the oleo fraction containing myrcene compounds, gum fractions A and B containing sugars and resin fraction containing sterols³. The ethyl acetate soluble portion constitutes about 45% of the gum resin, the hexane phase material constitutes about 9% of the gum resin and consists essentially of the diterpenoids, besides a small percentage of cholesterol and other unidentified compounds. Material contained in the benzene phase constitutes about 14% of the gum resin²⁰. Mukolol, a new diterpene alcohol, had been determined and isolated from the gum resin of *C. wightii*²¹.

Since alkaloids and quassinoids of *C. wightii* have not been studied extensively by these standard techniques, an effort should be made in this direction. The arduous and longstanding sporadic attempts in defining the principles of trying to find plant extract efficacies are in many ways incomplete. Some of these principles can even include the subjective but to some extent justified

beliefs of tribals and non-allopathic methods of treatments. They need to be reviewed and correlated with better defined standards and refined further to include biologically active fraction with their characteristics.

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Determination of hypoglycemic activity in *Morus indica* L. (mulberry) shoot cultures

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The aqueous extract from the leaves of shoot cultures of two elite varieties of mulberry, viz. S-34 and S-36, showed the presence of hypoglycemic activity when fed to the diabetic rats. The hypoglycemic activity of the extracts of leaves from the shoot cultures was higher and better as compared to the field-grown plant leaves. Based on these results, the usefulness of *in vitro* plantlets of mulberry, as a source of antidiabetic compound (S) is discussed.

MEDICINAL plants and their extracts have gained increasing importance over the past several years, since they can be used as a source for the preparation of herbal drugs¹. Studies on *in vitro* shoot cultures of medicinal plants have shown the presence of known and new pharmacologically active compounds².

Mulberry is valued for its foliage which constitutes the chief feed for silkworms. Besides its use in silk industry, mulberry leaves are useful as cattle fodder. They are nutritious and palatable and are known to improve milk yield when fed to dairy animals³. Analysis of mulberry leaves collected from different parts of India has revealed the presence of proteins, carbohydrate, calcium, iron, ascorbic acid, β -carotene, vitamin B-1, folic acid and vitamin D. All these are essential for the growth of the silkworm⁴.

Apart from their use as animal and insect feed, mulberry leaves have been shown to possess medicinal applications as they contain diuretic, hypoglycemic and hypotensive agents³. Thus, non-toxic mulberry leaves would be an ideal source for testing the presence of hypoglycemic activity. The present report describes results of the presence of hypoglycemic activity in extracts of leaves from *in vitro* shoot cultures and field-grown plants of mulberry.

Shoot cultures of mulberry (*Morus indica* L. var. S-34 and S-36) were used for experiments. The cuttings from mature trees of these two varieties were collected from Central Silk Research and Training Institute, Mysore, and were planted in experimental station at Trombay, Mumbai. These two varieties were selected on the basis of their good quality foliage throughout the year, their amenability to vegetative propagation with fast regenerative capability, high nutritional value and more resistance to stress, diseases and pests⁵. From such field-grown plants, buds which sprouted within 4–6

weeks were used for the establishment of shoot cultures. Auxiliary buds were surface-sterilized with 0.1% HgCl_2 for 5 min. After rinsing 5–6 times with sterile water the auxiliary buds were cultured aseptically on medium⁶, supplemented with IAA ($5.71 \mu\text{M}$) containing 3% wt/vol sucrose on which they produced extensive shoots and roots. The detailed protocol for establishment of shoot cultures from such plantlets has been described earlier⁷. Among these two varieties, S-36 grew very well on solid medium (i.e. MS supplemented with $22.10 \mu\text{M}$ BAP), whereas S-34 variety produced ample shoots in liquid medium of the same composition. From a single cultured auxiliary bud of S-34, within a period of three weeks 30–35 shoots were formed. The pH of the medium was adjusted to 5.8 before gelling the medium with 0.8% agar (HiMedia, 201 HiMedia Laboratories Pvt Ltd, Mumbai, India). Both solid and liquid cultures were maintained in a culture room at $25 \pm 2^\circ\text{C}$ at a relative humidity of 50–60% and were exposed to continuous fluorescent light (ca. 1000 lux). The liquid cultures were kept on a shaker at 70 rpm for continuous agitation.

The leaves from field-grown S-36 or S-34 variety were cleaned with water, blotted, weighed and homogenized in ice-cold water, first in mixer-grinder (Sumeet Company, Mumbai, India) and then in Tri-R Stir-R homogenizer (model S63C, Tri-R Instruments Inc., NY, USA). Similarly leaves from shoot cultures of S-36 or S-34 variety growing in test-tubes were taken, weighed and homogenized. All extracts were filtered through nylon wire mesh and the aqueous suspensions, having pH 6.1 to 6.4, were used for the bioassay.

Normal male Wistar rats weighing 190–210 g maintained on stock laboratory diet were used. The diet constituted 70% wheat, 20% bengal gram, 4% yeast, 5% fish meal, 0.75% til oil, 0.25 shark oil (corresponding to 60% carbohydrate, 30% protein, 5% fats/oils, 5% fibre), supplemented with vitamins and minerals mixture.

Diabetes was induced in rats by a single intramuscular injection of streptozotocin (60 mg of STZ/kg body wt) after 24 h of fasting⁸. The diabetic animals were stomach-fed with extracts using catheter tubes over a period of 3 days.

The blood was collected from tail-vein before feeding, 2.5 h after first feeding and after 5th feeding on the 3rd day. During this period (otherwise also), rats were maintained on the diet stated above. The blood was deproteinized⁹ and glucose was estimated immediately by Glucofix kit¹⁰ (Minarini Diagnostics, Italy) in protein-free supernatant. The hypoglycemic activity is expressed as percent reduction in blood glucose. Data was subjected to statistical analysis using Student's *t* test.

The aqueous extracts of mature leaves from field-grown S-36 variety exhibited hypoglycemic activity in STZ-diabetic rats within 2.5 to 3 h as shown in Table 1. The extent of reduction in blood glucose level decreased

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Table 1. Hypoglycemic activity in leaf-extracts of S-36 and 34 varieties of *Morus indica* grown in field or in cultures

Source	Maturity	Dose/feed	n	Reduction in blood glucose (%)		Reduction/g (%)*	
				2.5 h	3rd day	2.5 h	3rd day
S-36							
FGL	Old	250	15	26.5 ± 1.2	14.9 ± 1.4	21.2	11.9
FGL	Young	260	11	nil	nil	—	—
TCL	Old	130	13	16.6 ± 1.4	26.0 ± 1.2	25.6	40.1
TCL	Young	200	11	nil	nil	—	—
S-34							
FGL	Old	300	11	37.7 ± 1.2	9.3 ± 0.9	25.1	6.2
TCL	Old	700	15	34.4 ± 1.3	40.2 ± 1.1	49.2	57.5

The dose is indicated as extract corresponding to mg of leaves/feed/animal. Total of five feeds were given. Diabetic rats were 8–10 post Stz-days. Zero hr blood glucose were exceeding 550 mg/dl and were taken as control. *P* values were <0.005 for S-36 and <0.001 for S-34 variety compared to zero hr blood glucose. FGL: field- grown leaves; TCL: leaves of shoot cultured plants; Young leaves – weight < 1.0 g/leaf. Old leaves – weight > 1.5 g/leaf.

*per gram of leaves.

on the 3rd day in spite of giving feeds twice daily. This partial loss of hypoglycemic effect could possibly be attributed to rapid metabolism of the compound(s) responsible for the observed effect. However, the extract of young leaves from field-grown S-36 variety did not show any effect. The presence or absence and concentration of any bioactive compound are known to be influenced by the age of tissue, age of plant, season and location or area of plant. The difference in hypoglycemic activity of old and young leaves in the present study, could be attributed to the age of leaves. Nearly half the dose of leaves from cultured plantlets of S-36 variety caused 16% reduction in blood glucose after 2.5 h which increased to 26% on the 3rd day.

The leaves from field-grown S-34 variety showed 38% reduction in blood glucose after 2.5 h which decreased to nearly 9% on the 3rd day (Table 1). This decrease was similar to that observed with extract of S-36 variety leaves stated above. It can be seen that feeding 700 mg of extract of leaves of S-34 shoot cultures resulted in 34% reduction in blood glucose after 2.5 h which increased to 40% after 3 days feeding.

The total number of feeds were the same for all the groups, though the amount in each feed varied. Therefore, comparative hypoglycemic efficacy per gram of field-grown and *in vitro* shoot cultured leaves is given in Table 1. It is evident that the response of leaves from shoot cultures of both S-34 and S-36 was better than field leaves possibly due to the *in vitro* and *in vivo* stability of compounds responsible for blood glucose reduction. The retention of hypoglycemic response up to 3rd day in case of leaves from shoot cultures is in contrast to that observed for leaves from field grown plants of the same variety. This could presumably be ascribed to the differences in the nature and/or composition of hypoglycemic compound(s) present in natural/field grown plant and cultured plantlet(s).

The foregoing observations bring out for the first time the presence of hypoglycemic activity in field-grown mulberry leaves using diabetic rats as a model for testing. Moreover, hypoglycemic activity from cultured leaves appeared to have better efficacy. It is difficult to compare the efficacy of the extract used in the present studies with the known sulphonyl ureas employed in practice. Nevertheless, a gross comparison can be made. Feeding of chlorpropamide (Diabenese, Pfizer Company) mixed in a diet at a dose of 90–100 mg/rat/day for 5 consecutive days resulted in 50% reduction in blood sugar in STZ-diabetic rats⁸. However, feeding of 700 and 130 mg extract of leaves from plantlets of S-34 and S-36, for 3 days caused 40% and 26% reduction in blood glucose respectively. In fact, the efficacy may be significantly more after purification of the compound(s) from the extract. These studies are underway.

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Use of revegetated coal mine spoil as source of arbuscular mycorrhizal inoculum for nursery inoculations

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The present investigation examines the potential use of revegetated coal mine spoil as a source of arbuscular mycorrhizal inoculum for inoculating nursery seedlings. Rhizosphere soils of five tree species were used as sources of mycorrhizal inoculum. Soils contained seven spore-forming species of AM fungi. The substrate used in the pot experiment was a mixture of unsterilized coal mine spoil (without any mycorrhizal propagule) and autoclaved sandy loam soil. *C. siamea* and *D. indica* were used as the test plants. Measurements were made of shoot and root biomass, P uptake, per cent mycorrhizal infection and spore population of AM fungi. Growth measured as shoot and root dry weight was significantly higher in seedlings inoculated with soil inoculum from under *D. sissoo*, *C. siamea*, *D. indica* and *A. indica*. *A. scrobiculata* was found to be the best fungus in terms of root colonization ability and effectiveness to promote P uptake and growth in plants. A consistently poor growth response of the seedlings to soil inoculum from under *E. hybrid* was due to the ineffective association formed by *G. geosporum*. Whilst spores of *S. calospora* were not present in the rhizosphere soils of *D. indica*, they were formed in *C. siamea* pots inoculated with the same soil. This indicates that *S. calospora* also persisted in the soil in the form of propagules other than the spores. The results of this study justify the use of revegetated coal mine spoil as an effective and economical source of endomycorrhizal inoculum for inoculating nursery seedlings.

REVEGETATION and reclamation of mine spoils has been problematic because of the poor physical conditions, extremes of temperature and pH, low levels of organic and inorganic nutrients, toxic levels of heavy metals and lack of beneficial micro-organisms. Spreading top soils, a source of some beneficial organisms before revegetation may be helpful in establishing vegetation on mine spoils¹, but this has met with limited use because of the high costs of transportation of top soils. Reclamation of mined lands, therefore, requires innovative approaches that reduce the cost and increase the chances of success of plant establishment and survival². Plant establishment on mine spoils can be facilitated by endomycorrhizas formed by arbuscular mycorrhizal (AM) fungi³, as they are particularly effective in making positionally unavailable nutrients available through greater exploration of soil volume⁴. Introducing mycor-

rhizal fungi in freshly stockpiled overburden spoil would require planting of nursery seedlings inoculated with propagules of AM fungi. Use of this method will be most effective if plants are inoculated with AM fungi which are known to form mycorrhizal associations on mine site⁵. Differences in AM endophytes in their ability to colonize roots and improve growth⁶ and P uptake⁷ in plants have been observed. Native AM fungi have been found to be more effective than introduced fungi in improving the plant growth^{8,9}. Studies on growth responses of different host species to AM fungi in coal waste¹⁰ have concluded that research on value of endomycorrhizas to survival and growth of plants should be concentrated on testing a variety of endophytes which have persisted on the bituminous coal mine spoil, in order to find an ecologically adapted AM endophyte.

AM fungi are obligate symbionts and artificial medium for their independent growth has not been identified yet. Plants must be inoculated with inoculum produced on living roots in open pot cultures. Production of large amounts of mycorrhizal inoculum in pots for large-scale nursery applications is not economically and practically feasible. Rhizosphere soils of different tree species colonizing old mine spoils at the mine site, may prove to be valuable sources of inoculum of ecologically adapted strains of AM fungi². In order to use revegetated mine spoil successfully, it is necessary to test its effectiveness as a source of mycorrhizal inoculum on different plant species. Moreover, an understanding of how individual fungi colonize roots, survive and effect P uptake from mixed populations on revegetated mine spoils will also be valuable in assessing the contributions of AM fungi under nursery conditions¹¹.

The aim of this study was to examine the potential use of revegetated coal mine spoil from under different tree species as a source of endomycorrhizal inoculum for inoculating seedlings of two nitrogen-fixing tree species, *Cassia siamea* Lamk. and *Derris indica* (Lam.) Bennet. suitable for restoring degraded tropical areas.

The substrate used for the pot experiment was a mixture of unsterilized coal mine spoil obtained from the freshly stockpiled overburden at Jayant open cast mine site (E 82°36'40"–82°41'15" and N 24°6'46"–24°11'5") of Northern Coalfields Ltd, Singrauli, India and autoclaved sandy-loam soil (2 : 1 v/v). Bioassay test using corn (*Zea mays* L.) plants showed that the coal mine spoil had no mycorrhizal propagules. The substrate soil had the following chemical properties: pH, 6.2; OC, 1.33%; EC, 0.27 dsm⁻¹; P, 2.2 mg kg⁻¹; K, 46.2 mg kg⁻¹. Five kg of air-dried soil was transferred to each 20 cm wide and 14 cm high clay pots, watered thoroughly and allowed to drain for 1 week. *C. siamea* and *D. indica* seeds were surface-sterilized in 10% solution of sodium hypochlorite for 2 min, soaked in sterile water for 24 h and sown in pots containing sterilized sandy-loam soil.

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Rhizosphere soils (20 cm below soil surface) of five tree species, *Dalbergia sissoo* Roxb., *Cassia indica* A. Juss. and *Eucalyptus* hybrid growing on a 5-year-old reclaimed overburden at Jayant coal mine site, were used as sources of mycorrhizal inoculum. Soil samples were kept at room temperature¹² for one month before use. Rhizosphere soils were subjected to wet sieving and decanting¹³ and spores were collected and counted on grids drawn on filter paper. Spores were identified to species using current taxonomic guide¹⁴ and original species descriptions. Spore wall characteristics were examined at $\times 1000$ magnification using stains and Melzer's reagent. Spellings of scientific names of mycorrhizal species are those suggested by Almeida¹⁵. Seven spore-forming AM fungi, *Acaulospora scrobiculata* Trappe, *Glomus geosporum* (Nicol. & Gerd.) Walker, *Glomus aggregatum* Schenck & Smith emend. Koske, *Glomus micr aggregatum* Koske, Gemma & Olexia, *Scutellospora calospora* (Nicol. & Gerd.) Walker & Sanders, and undescribed species of *Acaulospora* (AY) and *Gigaspora* (GiB) were present in the rhizosphere of different tree species (Table 1).

Forty grams of soil inoculum, containing resting spores, infected root fragments and mycelia was placed about 6 cm below the soil surface in each mycorrhizal pot. Thirty-day-old uniform seedlings of *C. siamea* and *D. indica* were planted in mycorrhizal and non-mycorrhizal pots at the rate of one seedling per pot. For each host species, there were five treatments and each treatment was replicated three times. Pots were kept in the greenhouse in a randomized block design and watered daily as necessary. The temperature during the experiment ranged from 20 to 35°C and the photoperiod was 13 h.

Seedlings were harvested 180 days after transplanting. Dry weight of shoot and root was determined after drying samples at 70°C for 96 h. Phosphorus content was determined after digesting dried plant parts in tri-acid mixture containing $\text{HNO}_3 : \text{H}_2\text{SO}_4 : \text{HClO}_4$ (10 : 1 : 3) and analysing by molybdo-phosphate method¹⁶. Roots were examined at $\times 100$ –400 magnifications for the presence of infection after clearing with 10% KOH, acidifying in dil.HCl and staining in 0.01% acid fuchsin in lacto-

phenol¹⁷. Darkly pigmented roots were immersed in an alkaline solution of H_2O_2 until bleached¹⁸. Fifty 1 cm root segments, randomly collected from each plant species, were scored for the presence or absence of infection using slide method for assessing percentage mycorrhizal infection¹⁹. Spores were identified to species and counted by the method described above. Data were analysed using one-way analysis of variance.

Shoot and root dry weight and tissue P concentration in *C. siamea* plants were significantly increased in mycorrhizal pots inoculated with soils from under *D. sissoo*, *C. siamea*, *D. indica* and *A. indica* relative to uninoculated control. Plants inoculated with *E. hybrid* soils showed no significant difference in shoot and root dry weight and tissue P concentration relative to uninoculated control. Among mycorrhizal treatments, significantly higher shoot and root dry weight and shoot P uptake was observed in pots inoculated with *D. indica* soils. Arbuscular mycorrhizal infection was significantly higher in plants inoculated with the rhizosphere soils of *D. indica* (Table 3). Spores of *S. calospora* were present in pots inoculated with *C. siamea* and *D. indica* soils. Maximum number of spores (27) was observed in the pots inoculated with rhizosphere soils of *D. sissoo*. Lowest percentage of mycorrhizal infection (48) was observed in pots inoculated with *E. hybrid* soils.

Mycorrhizal inoculation with rhizosphere soils of *D. sissoo*, *C. siamea*, *D. indica* and *A. indica* significantly improved shoot and root dry weight and tissue P concentration of *D. indica* over uninoculated control. Shoot and root dry weight and tissue P concentration in plants inoculated with *E. hybrid* soils showed no significant difference relative to control. Among mycorrhizal treatments, significantly higher increase in root dry weight and shoot and root P concentration was observed in pots inoculated with *A. indica* soils. There was no significant difference in per cent mycorrhizal infection in plants inoculated with soil inoculum from under *D. sissoo*, *C. siamea*, *D. indica* and *A. indica* (Table 5). Maximum number of total spores (100) was observed in pots inoculated with the rhizosphere soils of *D. sissoo*. Lowest mycorrhizal infection (67.6%) and total

Table 1. Mean number of spores of AM fungi in the rhizosphere soils of five tree species colonizing coal mine spoil at Jayant

Host species	No. of spores of AMF species/100 g dry soil							Total
	ASCB ^a	A. sp. (AY)	Gi. sp. (GiB)	LGSP	CCLS	LMAG	LAGR	
<i>D. sissoo</i>	224	156	2	4	—	—	—	386
<i>C. siamea</i>	48	—	—	7	6	12	—	73
<i>D. indica</i>	42	—	—	6	—	—	4	52
<i>A. indica</i>	58	—	—	—	—	—	—	58
<i>E. hybrid</i>	—	—	—	8	—	—	12	20

^aMycorrhizal species code as suggested by Perez and Schenck²⁹. A. sp. (AY) and Gi. sp. (GiB) are undescribed species of *Acaulospora* and *Gigaspora*, respectively.

spore population (6) were observed in pots inoculated with soil inoculum from under *E. hybrid*.

In general, rhizosphere soils of *D. sissoo*, *C. siamea*, *D. indica* and *A. indica* tested as AM inoculants on *C. siamea* and *D. indica* produced more than 40% increase in shoot dry matter production over uninoculated control. There was no relationship between per cent mycorrhizal infection and spore numbers. Spores or sporocarp of mycorrhizal species, *G. aggregatum*, *G. micraggregatum*, *Acaulospora* sp. (AY) and *Gigaspora* sp. (GiB) present in rhizosphere soils of different host species from revegetated mine spoil were not observed in mycorrhizal pots.

The study shows that whilst rhizosphere soils of *D. sissoo*, *C. siamea*, *D. indica* and *A. indica* acted as an effective source of arbuscular mycorrhizal inoculum, soils taken from under *E. hybrid* were not effective in enhancing plant growth. The AM fungal species, *A. scrobiculata*, not only effectively colonized root but also consistently increased P uptake and shoot dry matter production in both *C. siamea* and *D. indica*. Studies on competitive interactions between arbuscular mycorrhizal species have suggested that the fungal species that colonize root first, may be at an advantage over late colonizers²⁰. This may account for the widespread occurrence of *A. scrobiculata* in revegetated coal mine spoil, as is evident by its presence in the rhizosphere soils of all the tree species, except in *E. hybrid*. Significantly higher increase in growth and P uptake in *C. siamea* inoculated with *D. indica* soils was an effect

of higher root colonization due to the presence of both *A. scrobiculata* and *S. calospora*, as was evident by the presence of spores of the two mycorrhizal species in pots. Previous studies have also revealed that inoculations with mixed inoculum, containing more than one AM endophyte, resulted in higher root colonization and increased P uptake and growth in plants^{7,21,22}. Recently, increased growth in plants inoculated with dual inocula of AM fungi has been reported to be due to the increased transfer of P to the shoot from the root⁷. No significant change in shoot biomass and P uptake in plants inoculated with *E. hybrid* soils, relative to uninoculated control, was possible due to the constant balance maintained in carbon or P demand between the source (host) and the sink (fungus). *G. geosporum* associated with plants inoculated with *E. hybrid* soils was not effective in increasing P uptake and shoot biomass.

From this study, it is evident that whilst *C. siamea* support growth and reproduction of both *A. scrobiculata* and *S. calospora*, *D. indica* favours growth of *A. scrobiculata* only. This supports the works showing that host plants can be selective in the growth and reproduction of certain AM fungal species under a particular set of environmental conditions^{22,24}. Apparent host specificity may, however, occur if host susceptibility does not coincide with the propagule infectivity⁵. Spores of *S. calospora* were not present in *D. indica* soils used as inoculum, but were formed in mycorrhizal pots of *C. siamea* inoculated with the same soil. This indicates

Table 2. Dry weight and phosphorus concentration of *Cassia siamea* inoculated with soil inoculum from under five tree species

Soil inoculum source	Shoot dry wt (mg/plant)	Root dry wt (mg/plant)	Shoot P conc. (%)	Root P conc. (%)
<i>D. sissoo</i>	1.63 ^b	0.71 ^b	0.21 ^b	0.15 ^b
<i>C. siamea</i>	1.19 ^b	0.51 ^{bc}	0.15 ^b	0.15 ^a
<i>D. indica</i>	2.23 ^a	1.03 ^a	0.5 ^a	0.14 ^a
<i>A. indica</i>	1.0 ^b	0.3 ^c	0.15 ^b	0.14 ^a
<i>E. hybrid</i>	0.34 ^c	0.08 ^d	0.07 ^c	0.06 ^b
Control	0.59 ^c	0.18 ^d	0.04 ^c	0.09 ^b

In each column, the mean values superscribed with the same letter do not differ significantly ($P = 0.05$).

Table 3. Comparison of percentage infection and spore numbers in *C. siamea* inoculated with soil inoculum from under five tree species

Soil inoculum source	Mycorrhizal infection (%)	Spore number of AMF species/100 g dry soil			
		ASCB	LGSP	CCLS	Total
<i>D. sissoo</i>	75 ^b	27	—	—	27 ^a
<i>C. siamea</i>	76 ^b	8	—	3	11 ^c
<i>D. indica</i>	85 ^a	11	—	9	20 ^b
<i>A. indica</i>	58 ^c	21	—	—	21 ^b
<i>E. hybrid</i>	48 ^d	—	3	—	3 ^d

In each column, the mean values superscribed with the same letter do not differ significantly ($P = 0.05$); — = absent.

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Table 4. Dry weight and phosphorus concentration of *Derris indica* inoculated with soil inoculum from under five tree species

Soil inoculum source	Shoot dry wt (mg/plant)	Root dry wt (mg/plant)	Shoot P conc. (%)	Root P conc. (%)
<i>D. sissoo</i>	2.53 ^a	2.06 ^b	0.11 ^b	0.09 ^b
<i>C. siamea</i>	2.36 ^a	2.03 ^b	0.10 ^b	0.10 ^b
<i>D. indica</i>	2.71 ^a	2.15 ^b	0.10 ^b	0.10 ^b
<i>A. indica</i>	2.86 ^a	3.10 ^a	0.16 ^a	0.15 ^a
<i>E. hybrid</i>	1.42 ^b	1.53 ^c	0.07 ^c	0.07 ^c
Control	1.38 ^b	1.05 ^c	0.08 ^c	0.06 ^c

In each column, the mean values superscribed with the same letter do not differ significantly ($P=0.05$).

Table 5. Comparison of percentage infection and spore in *D. indica* introduced with soil inoculum from under five tree species

Soil inoculum source	Mycorrhizal infection (%)	Spore number of AMF species/100 g dry soil			
		ASCB	LGSP	CCLS	Total
<i>D. sissoo</i>	83.3 ^a	100	—	—	100 ^a
<i>C. siamea</i>	88.9 ^a	35	—	—	35 ^b
<i>D. indica</i>	84.6 ^a	36	—	—	36 ^b
<i>A. indica</i>	79.6 ^a	16	—	—	16 ^c
<i>E. hybrid</i>	67.6 ^b	—	6	—	6 ^d

In each column, the mean values superscribed with the same letter do not differ significantly ($P=0.05$); — = absent.

that *S. calospora* also persisted in the form of propagules other than resting spores. Previous studies have shown that the network of intra- and extra-radical hyphae or intra-radical spores act as important sources of AM propagules in dry soils^{12,20,25}. The variable amount of mycorrhizal spores present in the soils used as inoculum source had little effect on the percentage infection of roots at the final harvest. Gazey *et al.*²⁶ also found that the amount of *A. laevis* inoculum added had little effect on the proportion of roots colonized, once maximum percentage of root length colonization was reached. Therefore, it appears that the increasing number of infections with increasing inoculum density usually results from an increasing probability of infection by individual propagules of mycorrhizal species, rather than from increased energy. Maximum number of spores of *A. scrobiculata* were consistently produced in pots inoculated with rhizosphere soils of *D. sissoo*, which incidentally had the highest amount of spore inoculum. This supports the work of Gazey *et al.*²⁶ who also found that the number of spores produced in *A. laevis* differs with the inoculum quantity. Since the root system of *C. siamea* is relatively coarse with fewer root hairs than *D. indica*, it is plausible that a comparatively lower population of *A. scrobiculata* in *C. siamea* pots relative to *D. indica* pots was due to the differences in the root morphology²⁷.

Unsterilized coal mine soil was used as a substrate in the present investigation, as it provided 'natural

conditions' for the growth of ecologically adapted strains of AM fungi. Evidence of ecological adaptation in mycorrhizal fungi has been provided by several workers^{3,28}. The results of this study justify the use of revegetated coal mine spoil as an effective and economical source of endomycorrhizal inoculum from within the production system, but suggest the need to evaluate the specific effects of rhizosphere soils from revegetated overburden as sources of AM inoculum on individual host species prior to their utilization in large-scale nursery inoculations.

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INSA MEDAL FOR YOUNG SCIENTISTS – 1997

Instituted by the Indian National Science Academy in 1974 the Medal is awarded annually in recognition of outstanding work of scientists below the age of 32. Only those born on or after 1 January 1965 are eligible for consideration in 1997. The work done in India by the nominee will only be taken into consideration for the award.

The awardee is presented a medal, a certificate, and a cash award of Rs. 25,000. In addition, the recipient may be considered for a research grant up to Rs 5 lakhs for a period of three years. Preferential consideration will also be given for attending conferences/pursuing collaborative research under bilateral exchange programme with overseas Academies. An awardee, who is unable to obtain a suitable placement, will be considered for an interim Fellowship.

A candidate may only be nominated once. However, a nomination will remain valid for consideration for 3 years or until the age of eligibility whichever expires earlier.

Nominations for the awards for 1997 may be made by Fellows of the Academy, previous recipients of INSA Medal for Young Scientists as also by the established scientific societies of all India character, University faculties and departments or the research institutions. Last date for the receipt of nominations in the Academy is 15 November 1996.

Nomination proforma can be obtained from Dr Alok Moitra, AES (Council), Indian National Science Academy, Bahadur Shah Zafar Marg, New Delhi 110 002 by sending a self-addressed envelope of 25 cm × 12 cm size.

BOOK REVIEWS

The Collected Papers of Albert Einstein: Volume 1 – The Early Years, 1879–1902; Volume 2 – The Swiss Years: Writings 1900–1909; Volume 3 – The Swiss Years: Writings, 1909–1911. English Translation. Princeton University Press, Princeton, New Jersey – a combined review.

When Albert Einstein died in April 1955 at the age of seventy-six, his estate passed into the joint custody of his long-time secretary Helen Dukas, and his close friend Otto Nathan who was named sole executor. Making Einstein's writings and papers – especially his collected scientific works – widely available to the community of scholars has taken an inordinately long time, not merely because of the volume of material involved. Under the joint sponsorship of the Hebrew University of Jerusalem, where the Einstein Archive of 43,000 documents is housed, and Princeton University Press, and with John Stachel of Boston University as overall Editor, a monumental series of about forty volumes – the Documentary Edition – started appearing in the late eighties. Simultaneously, under a separate project supported by the National Science Foundation of the USA, Princeton University Press is making available a companion series of volumes containing English translations of the original volumes, specially prepared by Anna Beck with the assistance of Peter Havas and Don Howard. Some volumes in the series contain general correspondence during the various phases of Einstein's life, while the others contain his scientific papers in the same periods. The present review looks at volumes 1, 2 and 3 of this latter project, while a review of the later volume 5 has appeared earlier in *Current Science* (1995, 69, 882).

The prefaces to these volumes clearly state that they do not stand on their own, but must be taken as supplementing the more comprehensive Documentary Edition which contains material in the original languages, with numerous footnotes and explanations, which are here omitted. This robs these volumes of their value only slightly, though one misses some of the presumably illuminating cross-references and editorial comments.

Volume 1 is devoted to correspondence during the years 1879 to 1902 – from Einstein's birth to age twenty-three. More

than half the letters included here were apparently discovered by the editors while preparing this volume, and this includes over fifty letters between Einstein and his fellow student and first wife Mileva Maric. The volume begins with an extract from a biographical sketch of Einstein by his younger sister (and only sibling) Maja, written with sisterly affection. It covers the history of the family over a couple of generations, and the ill-starred business adventures of the father Hermann Einstein who ultimately died early really unaware of his son's extraordinary powers. Maja sees her mother Pauline nee Koch with understandable fondness, and talks of the qualities of perseverance, concentration and love of music evident in Albert already at a young age. 'Only persistence that does not rest until all that is unclear is eliminated and all difficulties are overcome allows an idea to take shape and be recognized as truly one of genius.' He was given to violent tantrums as a young child but these soon disappeared. Maja credits Pauline for Albert's affinity for music, and Hermann for his love of logical thinking and mathematics. After the parents' move to Italy in 1894, in search of better fortunes, Albert became acutely unhappy with the rigid curriculum at the Luitpold Gymnasium in Munich – we remember that a generation later a similar school produced Werner Heisenberg! Young Albert ran away from school to Italy, then spent a year at a more congenial cantonal school in Aarau in Switzerland, and vowed to make it on his own to study at the prestigious Federal Institute of Technology – ETH – in Zurich. In this he was supported by his parents. This excerpt ends in 1896, about the time Albert was ready to enter the ETH as a student of mathematics.

From the rest of volume 1, we get already a clear picture of what was to come: his great admiration for the writings of Kirchhoff, Helmholtz and specially Boltzmann; and his early thoughts and preoccupation with problems of electrodynamics of moving bodies, the problem of the ether, with the Planck radiation law and the photo effect of Lenard, the foundations of thermodynamics, and molecular motions and sizes. There are also grade sheets from various exams, and the lengthy encounter with the Swiss bureaucracy in connection with his application in 1899 for Swiss citizenship granted in

1901. Good to see that these wheels grind slowly always and everywhere! There is a touching letter from Einstein's father to his teacher and guardian Jost Winteler at whose home he stayed while in Aarau: 'It is a great relief to know that my son is under such loving care which is not only concerned with his physical well-being but also promotes his intellectual and inner life in such a noble fashion. At this young age the heart is most receptive to a good model and I am convinced that your good influence will leave a lasting effect.' A brief – almost inevitable – adolescent affair with the Winteler daughter Marie ended by 1897. Then begins the exchange of letters with Mileva Maric, his fellow student at the ETH during 1896–1900. Gradually one sees the intensity of feelings grow and find expression. What does strike one is his generally optimistic and sunny disposition even in the most adverse circumstances, while she tends to be introverted and given to moods of depression and apprehension on various counts. Also there are remarkably few letters from Mileva to Albert – perhaps irretrievably lost?

At the ETH the mathematician Hermann Minkowski taught Einstein no less than nine courses over the four-year period. He also attended a course on Kantian philosophy. Detailed class notes on a 1897–98 physics course by Weber are included. While figures are unfortunately missing, it is interesting to see what such a course encompassed a century ago – heat flow and thermal conductivity, the nature of heat, Carnot's theorem and the Second Law; and some material on static and current electricity, with a good deal of experimental detail to accompany the theory.

An early 1898 letter from Albert to Maja recalls their parents' sad state and his inability to help them materially. Then already in 1899 in a key letter to Mileva he says the ether is problematic and that electromagnetic waves propagate in empty space with no carrier: 'I am more and more convinced that the electrodynamics of moving bodies, as presented today, is not correct, and that it should be possible to present it in a simpler way. I think that the electric forces can be directly defined only for empty space, (which is) also emphasized by Hertz.' The initial friendly but formal letters to Mileva turn more warm and intimate; but at the same

time there are references to family tensions and his parents' profound unhappiness at this friendship. Now and then we see expressed Albert's feelings of frustration and happiness denied, but a faith that the future will make amends: 'But destiny seems to bear some grudge against the two of us. But this will make things all the more beautiful later on, when all obstacles and worries have been overcome.' The earliest reference to his interest in the photo effect and Lenard's experiments is in a May 1901 letter, about the same time he expresses his being not fully convinced by Planck's work on radiation. On the employment front, there were many enquiries but few opportunities, and for a while Albert and Mileva lived a hand-to-mouth existence with temporary 'leave vacancy' teaching positions. Despite all these difficulties his intense commitment to scientific problems and independence of thought are always evident. The offer of the post of 'Technical Expert Class 3' at the Patent Office in Bern in June 1902 – with the timely help of a good friend – finally brings freedom from financial worry, and the chance to devote himself 'in his spare time' to his true interests.

Turning next to volumes 2 and 3, these contain Einstein's scientific writings during the periods 1900–1909 and 1909–1911 respectively. From 1902 to 1909 he worked at the Patent Office, then from 1909 to 1911 as an Associate Professor at the University of Zurich. There was then a brief move to Prague, followed by a return to Zurich as a professor at the ETH. This did not last long, as in early 1914 he made the move to the Prussian Academy of Sciences at Berlin. Thus ended his Swiss period. Towards the end of volume 3 there are some papers written from Prague. In spite of the declared shortcomings of these volumes they are an unbelievable treasure for every serious student of physics.

The earliest papers in volume 2 deal with problems of capillarity, intermolecular forces and ways of parametrizing them. Then appear three fundamental papers on statistical mechanics, which are however not very well known. The first of these was submitted in June 1902, evidently exactly one week after receipt of the letter of appointment at the Patent Office. In this paper Einstein declares that his aim is to close the gap, in the foundations of thermodynamics, between mechanics

and probability, and arrive at the laws of entropy, thermal equilibrium and the Second Law. These were his own independent approaches to these very basic problems; while he was deeply influenced by Boltzmann's work (in many letters to Mileva he asks for 'my copy of Boltzmann'), it turned out that Gibbs' work was not available to him. Indeed in a short note of 1910 reproduced in volume 3, Einstein remarks: 'Had I been familiar with Gibbs' book at that time, I would not have published those papers at all, but would have limited myself to the discussion of just a few points.' These papers are written in a not too transparent style – compared of course to his later masterpieces. But without doubt careful study of each of them, even almost a century later, would be most rewarding.

Now we come to the magic year 1905. So often have we been told, and so often have we told others, that this was a year of miracles for physics. In volume 2 we see why, and using the colloquial expression any decent physicist would gladly give an arm and a leg to have translations of the trio of 1905 papers next to her pillow – the 'photon paper' submitted on March 17th, the Brownian Motion paper submitted on May 11th, and the Special Relativity paper sent in at the end of June. The thrill of reading the originals, even in translation, is indescribable. In each of these papers we find a vastly improved writing style (compared to 1902), and complete mastery of ideas and understanding. Already in the first paper deriving the existence of quanta of radiation from the Wien limit of Planck's radiation law, Einstein speaks of localization and indivisibility of quanta – '... when a light ray is spreading from a point, the energy is not distributed continuously over ever-increasing spaces, but consists of a finite number of energy quanta that are localized in points in space, move without dividing, and can be absorbed or generated only as a whole'. The application to explain the Lenard photo effect comes towards the end. The Brownian motion paper is designed to present convincing evidence for the reality of molecules and estimating their sizes. A few years later, in 1908, he presented a simpler account specially for chemists (Document 50 of volume 2). In between, his Ph.D. thesis submitted to the University of Zurich – again in 1905, dedicated to his close friend Marcel Grossmann, titled

'A new determination of molecular dimensions', and just eighteen pages long – is reproduced in its entirety.

The special relativity paper is the last of the three great works of 1905, and also the longest. The two postulates are stated boldly at the very outset, and it is stunning to see the majesty of the arguments unveiling the inevitable consequences. The analysis of time and the problem of simultaneity, the insistence on clearly given operational procedures for measurements, are as fresh to read today as ninety years ago. The first part deals extensively with space-time kinematics, the Lorentz transformation laws and their consequences, and even the twin paradox. He then proceeds to the application to the electrodynamic equations of Maxwell and Hertz, the Doppler effect, aberration, etc. The level and comprehensiveness of the exposition – in this very first paper – is unbelievable. Yet many years later Einstein would say that this work on special relativity was no comparison at all to the struggles he faced both with quantum theory and general relativity.

Many later papers come back to one or another point raised by these landmark works. The mass energy equivalence is given in a September 1905 paper. Volume 2 contains also a multitude of reviews of papers by others. Among his own master works we find here the 1906 paper applying the Planck idea to the problem of specific heats; and the December 1907 Jahrbuch review of special relativity towards the end of which he hints at the problem of gravitation. This fine sentence on the dispensability of the ether is well worth recall: '... electromagnetic forces appear here not as states of some substance, but rather as independently existing things that are similar to ponderable matter and share with it the feature of inertia'. We also remember that the Principle of Equivalence – 'the happiest thought of my life' – which formed the cornerstone of general relativity, came to Einstein while he was composing this review.

The final jewel in this volume is the text of the 1909 Salzburg Lecture wherein he analysed the energy fluctuation formula based on Planck's Law, and from this was led to the wave-particle duality for radiation – 'All I wanted is briefly to indicate... that the two structural properties (the undulatory structure and the

quantum structure) simultaneously displayed by radiation according to the Planck formula should not be considered as mutually incompatible.'

Volume 3 is somewhat different in character and content from volume 2. It covers the period 1909 to 1911 throughout which Einstein was at the University of Zurich. The major part of this volume is devoted to the lecture notes he prepared for three of his courses – mechanics taught during winter 1909–1910; kinetic theory of heat taught during summer 1910; and electromagnetism taught during winter 1910–1911. Elsewhere (in volume 5) Einstein writes that he enjoyed teaching a great deal. These lecture notes tend to be telegraphic and fragmentary, not in the style of his beautiful papers; yet they are valuable to see the organization of his thoughts and the way he conveyed them to students. Of the three sets of notes, the one on mechanics is relatively complete and coherent; while that on the kinetic theory of heat tends to be sketchy. Einstein goes to some effort to stress the nontrivial content of the principle of virtual work and d'Alembert's principle in dynamics, and many down-to-earth problems of mechanics are presented including rigid body kinematics and rotational motion. While the standard conservation laws are covered, the link to symmetry is not yet seen! The kinetic theory course emphasizes that molecular theoretic foundations can lead to all the basic statistical results for macroscopic systems. In the notes on electricity and magnetism, we see a sophistication in style and level of ideas, including this statement of the conception of a theoretical scheme as a totality and how it should be judged: 'We set up a conceptual system the individual parts of which do not correspond directly to empirical facts. Only a certain totality of theoretical material corresponds again to a certain totality of experimental facts.' These notes are also fairly well-organized, and have a practical feel about them. They go up to the Maxwell equations, but stop short of discussing the vector potential, the Lienard-Wiechert solutions, the gauge transformation idea, or a covariant formulation.

Aside from these three major items in this volume, there is a superlative 1910 review of special relativity, giving the historical origin of the ether concept, its problems, its demise, and then going on to his own work. A later 1911 Zurich

lecture is equally superb, but this time highlighting the role of Galilean invariance in mechanics and the need to retain it in electrodynamics. In the discussion that follows the lecture, Einstein handles all the searching questions with complete confidence, and at the end points out that special relativity is a restrictive concept and not a specific model for any phenomenon: 'The principle of relativity is a principle that narrows the possibilities; it is not a model, just as the second law of thermodynamics is not a model.' Some papers go back to the ideas of the 1905 'photon' paper and pose questions which at that time were of the greatest import: are quanta intrinsic to radiation or are they only linked to matter? Inter alia he refers to his 1905 calculations, and says in effect, 'I have shown that the Maxwell field on its own "must be quantized"'. What bold declarations and deep insights into phenomena, and of the directions in which future progress would have to go! In so many of his papers we see his grasp and mastery of statistical concepts – the manifold and often startling uses of the Boltzmann connection $S = k \ln W$, the exploitation of fluctuations – to tease out the implications of experimental observations, the limitations of theory. We see the power of his arguments from first principles, an uncanny gift for seeing far in advance of others the need for fundamental conceptual advances in so many directions. He had the ability not only to lead but to point to others the most promising directions.

Towards the end of volume 3 are two papers written – one suspects in a leisurely style – from Prague, one on molecular motions in solids and another on his first insights into the influence of gravity on light. Another major document is the extended 1911 Solvay Congress lecture on the specific heat problem. Here again Einstein expresses his conviction that the foundations of both mechanics and electrodynamics would have to be profoundly altered to take account of the quantum; it is staggering to read these lines from one who had done so much for the understanding of space and time. And the 'paradox' of wave particle duality for radiation is expressed again.

The declared limitations notwithstanding, every professional physicist will find these volumes (and surely the succeeding ones) of abiding value and interest. To

see how fundamental theories took shape and came into being, to perceive what led to them, to be face to face with documents which heralded the birth of many conceptions taken today for granted, to see that there was a time when they did not exist, and to view them condensing out of a mist and take on permanence after creation in Einstein's mind – these are experiences beyond value.

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Wild Edible Plants of India: Diversity, Conservation and Use. R. K. Arora and Anjula Pandey. National Bureau of Plant Genetic Resources, New Delhi. Price not stated.

From an initial phase of hunting and gathering to agricultural societies and finally to industrial economic societies, there has been progressive decline in the number of species on which human race has depended for food and other needs. In this process, while the biodiversity base became progressively smaller, bioproductivity per unit area and time increased dramatically. The chief reason was application of principles of genetics and plant/animal breeding. This resulted in the release of time for vocations other than growing food, and also led to increase in population in some parts of the world. However, there is now a realization that to make agriculture sustainable, wide genetic diversity base has to be combined with high bioproductivity. This is a major challenge before agricultural scientists and technologists.

Judging against this background, the book is both very timely and most welcome. This book is the sum total of life-long work of R. K. Arora on India's agri-biodiversity, its ancestors and the related species. Arora's special strength has been that he combines knowledge and experience of a professional botanist, ecologist and geneticist, all rolled into one. The authors, and NBPGR and ICAR deserve hearty congratulations for this

excellent and most unique work. In this venture Arora has been admirably assisted by Anjula Pandey. On all counts women, by their very nature and intuitively have been far more committed conservationists than men. In fact women as gatherers have played a very crucial role in domestication of crops/animals in the world at four nuclear centres of agricultural development in the Fertile Crescent, Southern Mexico, Andes and Huang Ho in China.

The book is a successor to an earlier book entitled *Wild Edible Plants of India* (1978) published jointly by the late H. B. Singh and R. K. Arora which was essentially a floristic enumeration of species diversity used for various plant parts. The present authors have not only vastly updated the earlier information base, but also added several altogether new and relevant material.

Part I of the book deals with the diversity of species, which have been enumerated on the basis of the useful plant parts namely, underground parts, leaves and shoots, buds and flowers, fruits, nuts and seeds. Species in each category have been listed alphabetically using their botanical name together with common names as far as possible. An account of each species includes its distributional range together with its use and floristic and ethnobotanical information of the edible plant part used. Wherever possible, information on actual nutritive value has also been provided. The authors have also included very valuable ethnobotanical information regarding the use by local communities. This use has been

based on trial and error experiments carried over the years supplemented by conscious selection made by the communities. The results of such large scale trials are today available to the present generation free of cost. Regrettably such efforts are neither regarded as innovation nor are they patentable or awarded/rewarded.

The number of species included in this book is around 1000. These also include, foods used by local people (including the ecosystem people) in times of famine and excessive droughts in order to subsist in such conditions. In addition, over 150 wild edible lower plants (e.g. algae, fungi, lichens, pteridophytes and gymnosperms) have also been included.

Part II of the book deals with wild and domesticated diversity together with plants with future potential. In that sense, these are rather less known economic plants that have a potential to come into the mainstream of agriculture. Over 56 species have been listed and many of these species have definite potential for being used on a large scale. The broad categories are: roots and tubers, leaves, vegetable, flowers and buds, tropical-subtropical fruits, temperate fruits and seeds/nuts/kernels. This category also contains many unconventional plants. Most of these are already in use and have potential to be cultivated on a large scale. Special mention may be made of bamboos which have tremendous potential as a source of raw material for paper pulp and furniture industry and as building material.

Part III of the book is devoted to conservation concerns, involving both *in*

situ and *ex situ* approaches. In addition, technology for cultivation of some of the important species needs to be developed so as to lessen the demand from the natural populations and thus help in their conservation. Some incipient domesticates are with tribals and other ecosystem people. More often, these are treasure troves of useful genes. All these need to be brought under a conservation plan because the concerned people may no longer cultivate these for long and they may abandon them for more productive varieties.

This is an excellent book and contains wealth of information in a consolidated form. With this as the base, there is need to build up a data base. Equally important is to take a total view of and plan for long term *in situ* and *ex situ* conservation of these species. The various user Departments/Ministries in Government of India and concerned States in India need to evolve a National Action Plan with a long-term perspective. I have no doubt this book would be extensively used by professional botanists, breeders, biotechnologists, experimental evolutionists as well as grass-root workers. It is indeed a trend-setting work which needs to be followed by all biodiversity-rich developing countries in tropics and sub-tropics. The authors deserve hearty congratulations for this exemplary work.

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NATIONAL METALLURGICAL LABORATORY
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INDIAN COUNCIL OF AGRICULTURAL RESEARCH
Jawaharlal Nehru Awards for Post-Graduate
Agricultural Research—1996

The Indian Council of Agricultural Research invites applications from post-graduate students who have obtained their Ph D degrees during the year 1995 in India in Agriculture or Allied Sciences for Jawaharlal Nehru Awards for Post-graduate Agricultural Research—1996. There will be sixteen prizes of Rs 10,000 each for outstanding research work of the following nature:

- (i) which may have bearing on finding a solution to any important national problem in the field of agriculture/ animal sciences, and/or
- (ii) which is likely to have impact on increasing the production or improving the quality of any important crop, human nutrition or animal productivity or increasing the technological efficiency of any process of economic importance connected with agriculture.

Candidates shall be required to submit the following document along with application form through the head of the Institute from where the thesis has been obtained.

- (i) A copy of the Ph D degree certificate.
- (ii) A copy of the thesis submitted by them for the award of doctoral degree.
- (iii) A certificate from the guide of the candidate for Ph D degree stating the extent to which the work is the candidate's own contribution.
- (iv) Six copies of the synopsis indicating precisely and in concise terms the work done by the candidate.
- (v) Any reprint of papers published/presented based on the doctoral thesis.
- (vi) Six copies of the application form with complete address for correspondence with telegraphic address if possible.

Each candidate will be judged on the basis of the originality and the applied value of the investigations as revealed in the thesis submitted by him. In all matters relating to the award, the decision of the Council shall be final and no correspondence on this account will be entertained. The candidates who obtained their Ph Ds in 1995 only need apply. The prescribed proforma for applying for this award may be obtained from the Council on or before September 15, 1996.

Application with complete documents as mentioned above, addressed to Dr R. C. Maheshwari, Assistant Director-General (CSC), ICAR, New Delhi 110 001, should be sent so as to reach on or before October 15, 1996. The last date for candidates in the Andaman and Nicobar Islands, Lakshadweep, States/Union Territory in the North Eastern Region, Ladakh Division of J & K State and Sikkim is October 31, 1996. The award-winning thesis will be retained by the Council for record. In case the application is not accompanied by a copy of thesis and the required number of copies of synopsis, the application form is liable to be rejected at the screening stage.

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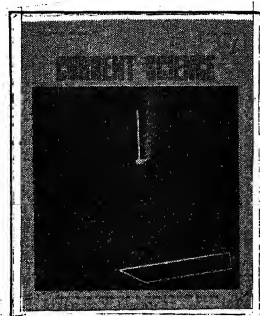
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In this issue

Controlling gene expression

The control of gene expression is primarily at the level of transcription, the step where information stored in the sequence of bases in DNA is transcribed into messenger RNA. The regulation of genes is then predominantly effected by the binding of specific proteins to control elements in the non-coding stretches of DNA.

Protein-DNA interactions are the molecular switches that modulate gene expression. Much of our present day understanding of gene expression dates back to the path-breaking work of Jacob and Monod, which led to the operon model. Several decades later the structures of protein repressors, the operator DNA segments and sometimes their complexes are available as a consequence of NMR or crystallographic studies. This wealth of molecular information promises to reveal the functioning of these regulatory switches at near-atomic resolution. Siddhartha Roy (page 100) describes biophysical studies on an important regulatory protein, the λ -repressor, which is crucial in switching the bacteriophage λ from the lysogenic to lytic mode of growth. Transcriptional control of gene expression is an area where the fusion of molecular genetics and structural biology has had a major impact.

P. Balaram

High energy physics

At the November 1995 meeting of the Indian Academy of Sciences, held in Madras, a special session was hosted by the Indira Gandhi Centre for Atomic Research, Kalpakkam. This was devoted to the

topic of 'High Energy Physics in the 21st Century'. This issue contains a special section (page 109) with written versions of many of the talks given, as it was felt by many that the theme and content would be appreciated by a wider audience.

The special section opens with G. Rajasekaran's overview, which itself introduces the articles that follow. It also states the basic driving force for high energy physics research. In spite of tremendous progress from the beginning of this century, our current basic idea of space and time is that given by Einstein and the quantum theory we use today is just a descendant of Planck's creation. But surely these ideas must give way to new ones as we probe shorter scales, and the only way to discover these new principles is to pursue higher energies, theoretically and experimentally.

D. P. Roy's article on the current very successful 'Standard Model' describes phenomena at energies less than a hundred times the rest energy of the proton, and correspondingly, length scales about a hundred times smaller than the size of the atomic nucleus. The very success of this model, which is by now rather complex with many parameters, raises the question of what lies at higher energies, and this is addressed in the next article by R. K. Kaul. Traditionally, much of this range has been regarded as unexplorable, but some of the daring ideas to reach such high energies are reviewed by A. Sen. R. Ramachandran sums up with the kind of questions one would like to answer, but with the cautionary note that such exercises in the past have missed the new surprises thrown up by the subject. In addition, he outlines a scenario for the development of the subject in our country.

R. Nityananda

A new look at an old concept

The idea of steric inhibition of resonance is well established and forms part of the standard organic chemistry curriculum. The concept is easy to understand. A substituent on a phenyl ring is generally twisted out of conjugation if two bulky groups are present in the ortho positions. Many examples are known which demonstrate this effect as well as its spectral and chemical consequences.

A more subtle effect is seen in anisoles and related molecules with a single ortho substituent. Instead of a partial reduction in conjugation, an enhancement was proposed by V. Baliah and M. Uma. They argued that in order to reduce steric repulsions, the methoxy group would remain in the plane of the phenyl ring, with the methyl group pointing away from the ortho substituent. Importantly, this preference was suggested to be greater than in the derivative without any ortho substituent (some degree of non-planarity is possible in the latter in view of the absence of steric interactions). This effect was called steric enhancement of resonance (SER). It represents one of the original contributions to stereo-electronic theory to be made from India.

The proof for SER was initially based on trends in dipole moments in a number of phenyl derivatives. Many additional spectral and reactivity features were also interpreted in terms of this effect. Since the magnitude of SER is usually small, results had to be interpreted with caution.

An attractive means of establishing and quantifying SER would be to use modern computational methods, for example by calculating structures and rotational barriers in representative systems. In a complimentary manner, S. R. Gadre and coworkers use the methodology they have perfected over the years to study the

problem of SER (page 130). Using *ab initio* MO wave functions on a number of model systems, the authors have examined the topological features of molecular electrostatic potentials. They find characteristic signatures in the MESP minima which reflect the inhibition of resonance in di-ortho substituted derivatives and also enhancement in mono-ortho counterparts. By placing an additional substituent at the para position, they have established consistency in the computed data.

J. Chandrasekhar

Perception of science

Support for the scientific enterprise depends greatly on the public per-

ception of science. This is especially true in the West but is becoming an increasingly important factor in India.

In an article reprinted in this issue (page 148), Martin Rees analyses the conditions under which discovery and invention flourish best. While his preoccupation with science in Britain may appear somewhat misplaced in this journal, readers would do well to ponder on many aspects of his analysis. Parallels can be readily drawn to the Indian situation, where science today does not have unqualified support and where attracting the best to scientific careers has become extremely difficult. The increasing pressure for research to yield visible dividends is transforming attitudes of scientists and those connected with organiza-

tions and funding of science. What are reliable indicators of success? Interestingly, public perceptions are rarely guided by utility. Martin Rees points out that 'irrelevant subjects' fascinate people most, with dinosaurs and cosmology topping the lists in Britain. In India the 'publish or perish' syndrome appears to have given way to the cry of 'patent or perish'. How do we create an intellectual climate for discovery and invention? How do we emphasize considerations that 'transcend the purely economic'. The article by Rees focusses on these and related issues and should provide a stimulus for future discussions.

P. Balaram

CURRENT SCIENCE

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CORRESPONDENCE

The Crest of the Peacock

I enjoyed reading a review by R. Sridharan of the book *The Crest of the Peacock, Non-European Roots of Mathematics*, written by G. G. Joseph and I felt I should convey a few remarks on the same. Sridharan's review (*Curr. Sci.*, 1996, 70, 753-754) was admirable and balanced with his generous comment on page 754: '... modern mathematics, as it is understood today, does owe a great deal to the renaissance in Europe, which in turn was a miraculous revival of Greek thought.' Even with regard to our own illustrious mathematicians' 'fundamental contributions to the study of quadratic indeterminate equations,...' and, in particular, (Jayadeva's) 'complete solution of quadratic indeterminate equations by the remarkable Chakravala method', the tendency for anyone to 'overstate his case' or soar, at times, to dizzy heights

of euphoria does perhaps require to be tempered in the light of what appears to be a very fair appraisal, in this connection, by André Weil in (page 24 of) Chapter I entitled 'Protohistory' of his well-known book *Number theory, an approach through history - From Hammurapi to Legendre*: '... For the Indians, of course, the effectiveness of cakravāla could be no more than an experimental fact, based on their treatment of a great many specific cases, some of them of considerable complexity and involving (to their delight, no doubt) quite large numbers. As we shall see, Fermat was the first to perceive the need for a general proof, and Lagrange, the first to publish one. Nevertheless, to have developed the cakravāla and to have applied it successfully to ... difficult numerical cases ... had been no mean achieve-

ment.' Sections IV, VI, VIII and IX of Chapter I of the above-mentioned book contain a detailed authoritative and objective analysis of the 'brilliant discoveries' from India - the kuttaka (=pulveriser) method "recalling to our mind Fermat's 'infinite descent'" the bhāvanā rules ('composition formulae' for special binary quadratic forms) and the 'cakravāla' (= 'the cyclic process') whose 'true originator remains unknown'.

S. RAGHAVAN

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Nobel prizes in X-rays or NMR

In the article '100 years of X-rays and 50 years of NMR' by R. Chidambaram *et al.* (*Curr. Sci.*, 1996, 70, 878-888), the authors have given a list of Nobel prizes won in the disciplines of X-rays or NMR. I feel that the following names should have been included in the table.

1. 1917: C. G. Barkla (Physics): Discovery of characteristic X-rays of elements.
2. 1936: P. Debye (Chemistry): Diffraction of X-rays and electrons in gases
3. 1946: H. J. Muller (Medicine): Pro-

duction of mutations by X-ray irradiation.

V. B. SAPRE

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Anti-hydrogen

1996 seems to be the year in which many experiments for which ultimate skills were required of physicists have fructified. Nellis and his group at Lawrence Livermore have metallized hydrogen (*Curr. Sci.*, 1996, **70**, 876-877); J. E. Baldwin and his group at Cambridge (*Astron. Astrophys.*, 1996, **306**, L13-L16) have made an important advance in optical astronomy. The resolving power for optical imaging of stars has improved from 1 arcsec to a few milliarcsec, by the technique of aperture synthesis; this may now make it possible to observe spots and other features on stars which could only be seen on the sun so far. Now comes the news on internet from CERN that antimatter has been created; specifically that nine atoms of anti-hydrogen have been 'observed'.

In 1898, Arthur Schuster the British physicist surmised the existence of

antimatter. With Dirac predicting the positron in 1929, Carl Anderson discovering it in 1932, Owen Chamberlin, Emilio Segre and others discovering the antiproton in 1955, it seemed just a matter of time before antihydrogen would be synthesized. Yet one had to wait more than 40 years for this to happen. Big machines like the low energy antiproton ring (LEAR) had to be built in which beams of positrons and antiprotons could be brought together in a cloud of inert xenon gas. The velocities of the antiprotons and the positrons slowed down to be almost the same so that one could capture the other and form a neutral atom.

The temperature too has to be kept below 14 eV (140000 degrees) so that the two component particles do not dissociate. When the antihydrogen atom is formed, being uncharged, it is not bent

by the magnetic field of LEAR, and it flies straight to the silicon detector. The experiment has only 'observed' the decay products of the antihydrogen atom, not the atom itself. The ultimate goal of isolating atoms of antimatter for further studies is still to be achieved. When this happens the comparison of the spectroscopy of the anti-protonic atom and the normal hydrogen atom may prove interesting from the point of view of testing weak violations of basic symmetries. The results of another experiment planned by Samuel Ting (of J particle fame) and others of detecting antimatter in space using an anti-matter spectrometer are eagerly awaited by cosmologists.

S. Ramaseshan, Raman Research Institute, Bangalore 560 080, India.

The 1996 Crafoord Prize

The Royal Swedish Academy of Sciences has awarded the 1996 Crafoord Prize in the biosciences with particular emphasis on ecology to Sir Robert M. May, University of Oxford, UK, for his pioneering ecological research concerning theoretical analysis of the dynamics of populations, communities and ecosystems. The value of the 1996 Crafoord Prize is US\$ 500,000.

Robert M. May, born in 1936 in Sydney, Australia, began his career as a theoretical physicist but has become, over the last twenty-five years, the person who has exerted the greatest influence on theoretical and empirical ecological research. Through his pioneering achievements, scientists now have a better understanding of the ecological dynamics of individual populations, the interplay between different species and whole ecosystems. Further-

more, his theoretical research has been of great significance for a number of important practical problems.

May's first great contribution concerned the hotly-debated question of whether stability is the cause of the diversity of ecosystems, or whether it is the other way round. May clarified that biological diversity does not automatically generate stability and that a number of different factors in combination lead to the stability of the ecosystem through various disruptions. May's book *Stability and Complexity in Model Ecosystems* (1974) brought about a drastic change of direction in the way of viewing the interaction between different species and the reaction patterns of the ecosystem. The book stimulated innovative research contributions in a number of different fields.

In the young science of nature conservancy biology, May's population dynamics models and theoretical analyses have come to play a fundamental role. This concerns, above all, mapping out what factors are most important for the survival possibilities of small and fragmented plant or animal populations in an environment that is exploited to an ever-increasing degree by man. Crucially, May's theoretical work has pointed at the significance of random events within the diminishing populations, with regard to their prospects of survival for longer periods.

Robert M. May is a professor at the Department of Zoology at the University of Oxford, England. He is also Chairman of the British government's Advisory Council for Science and Technology.

The 1996 Blue Planet Prize

The Asahi Glass Foundation has chosen the recipients of the Fifth Blue Planet Prize, an international award first given in 1992 at the United Nations Conference on Environment and Development in Rio de Janeiro. Presented annually to two recipients, the prize commends individuals, groups, and organizations whose achievements have contributed to the resolution of global environmental problems. The 1996 recipients are: Wallace S. Broecker of the United States, Newberry Professor of Geology at Columbia University, who has made major contributions to our understanding of climate change and global warming through his research into global ocean currents and ocean chemical cycles, particularly the carbon cycle; and the M. S. Swaminathan Research Foundation of India which is a nonprofit organization that supports the promotion of sustainable agriculture and rural development with methods that include the preservation and sustainable utilization of biodiversity, the improvement of soil and plant health using environment-friendly methods, and the creation of ecojobs for rural families. The research foundation, chaired by M. S. Swaminathan, is the first Blue Planet Prize winner from Asia. Each Blue Planet Prize winner will receive ¥50 million.

The M. S. Swaminathan Research Foundation (Madras, India) was founded in 1988 with the goal of promoting research and activism to further rural and agricultural development by environmentally sustainable and socially equitable means. The founder and chairman of this organization is M. S. Swaminathan, the recipient of the first World Food Prize, in 1987, and of the 1986 Albert Einstein World Science Award.

One of the research foundation's major achievements has been the study and conservation of coastal ecosystems, particularly mangrove wetlands. Based on its research into vegetation, soil salinity, and other aspects of mangrove habitats, the research foundation has taken steps to restore degraded wetlands. By promoting sustainable agroforestry and aquaculture, together with the use of organic fertilizers, the M. S. Swaminathan Research Foundation

helps establish integrated coastal management systems that can provide an ecologically sustainable livelihood for coastal families.

The research foundation also conducts a community biodiversity program to rescue endangered plant species from extinction, identifies microorganisms to serve as bioindicators of ecosystem health, and conserves genetic diversity of plant species used as food or in medicinal and other applications. This program includes the creation of a community gene bank to store collected seeds as well as the establishment of guidelines for conducting ecological observations. These are only a few examples of the many activities undertaken by the research foundation to help restore and maintain natural habitats and biological diversity through the application of technology.

In addition, the research foundation promotes the biovillage model of sustainable rural development in India, the People's Republic of China, and Southeast Asia. Further positive results from these efforts are expected in the future. By helping to conserve the natural environment of developing countries while supporting the economic viability of rural communities, the M. S. Swaminathan Research Foundation is playing an important role in the search for solutions to global environmental problems.

In 1990, the research foundation established as its core organization the Centre for Research on Sustainable Agricultural and Rural Development. MSSRF has also set up the Technical Resource Centre for the Implementation of the Equity Provisions of the Convention on Biological Diversity to gather information that will help rural families obtain the recognition and reward due to them for their past and present contributions to genetic conservation and enhancement. The programs of the research foundation are organized under the following five areas.

The first area consists of aligning environmental protection and agricultural productivity in coastal wetlands. In particular, the foundation conducts research into how to tie the ecological security of mangrove forests to the livelihood of coastal communities. With the

support of the International Tropical Timber Foundation, MSSRF conducted a survey of mangrove genetic resources throughout Asia and West Africa. The research foundation then developed a multimedia database and an international information service on mangrove ecosystems.

The second area involves research on the conservation of biodiversity, a fundamental requirement for sustainable agriculture. Specific activities include saving endangered plant species and habitats, promoting the revitalization of genetic conservation traditions of local peoples, and maintaining soil fertility by monitoring microorganisms in soil. The research foundation has also created a gene bank to provide modern techniques for the preservation and use of plant genetic material. Based on its research efforts, MSSRF helps set up sustainable agricultural systems.

The third area consists of the application of ecotechnology to sustainable agriculture. Ecotechnology is a term used to describe the blending of vanguard technologies, including biotechnology, with the ecological wisdom and practices of local communities so as to integrate the ecological and economic strengths of both approaches. Ecotechnology is put into action by the voluntary participation of whole communities, known as biovillages. Similarly, programs have been instituted to create ecojobs, which generate additional food and income from available natural resources in a sustainable manner. The United Nations Educational, Scientific and Cultural Organization (UNESCO) has designated MSSRF as the coordinator for the Asian Ecotechnology Network.

The fourth area is a program entitled Reaching the Unreached, which aims to bring the benefits of new technologies to the economically and socially disadvantaged, especially women and children, and promote gender equity in development.

The fifth area involves training programs and communications tools, including publications. Among its many other activities, the research foundation maintains databases, conducts educational programs, and holds workshops for policy makers and farming families.

Comments on the 'Principle of maximum physical hardness'

In a recent paper, Pearson¹ analyses the proof of Parr and Chattaraj² (PC) for the principle of maximum chemical hardness. He argues that the proof is very general and can also be applied to many observables, other than softness. In particular, using the equation (11) of PC (equation (1) below), he claims to prove the principle of maximum physical hardness. Thus two principles are claimed to be true—one of maximum physical hardness, and the other of maximum chemical hardness.

It is the aim of this letter to show that the arguments of Pearson¹ and those of PC² are in error. We have already commented³ on the paper of PC² and have given a numerical counterexample, which shows that their proof of the principle of maximum chemical hardness is in error. See also the recent paper of Chattaraj, Liu and Parr⁴ which says, 'Recently it has been correctly pointed out³ that the proof by two of us² of the maximum hardness principle is not true in general'. Here we point out why the method adopted by Pearson is incorrect.

As the derivation given by Pearson¹ is based upon that of PC, we consider the arguments given by them. They consider a non-equilibrium ensemble with the distribution $F(\mathbf{r}^N, \mathbf{p}^N)$ and denote the time-dependent expectation value of any observable A by $\bar{A}(t)$. That is,

$\bar{A}(t) = \int d\mathbf{r}^N \int d\mathbf{p}^N A(t) F(\mathbf{r}^N, \mathbf{p}^N)$. They claim that for a class of $F(\mathbf{r}^N, \mathbf{p}^N)$,

$$\bar{A}(0) = \langle A \rangle + \langle A \rangle^{-1} \langle (A - \langle A \rangle)^2 \rangle. \quad (1)$$

Equation (1) is just the equation (5) of Pearson² (equation (11) of PC), rewritten in a slightly different manner. A simple argument is enough to convince the reader that this equation cannot be correct. The left hand side of the equation depends on the non-equilibrium ensemble that one chooses while the right hand side has only equilibrium expectation values in it! In fact, the result of PC is valid specifically for the ensemble, $F(\mathbf{r}^N, \mathbf{p}^N) = \langle A \rangle^{-1} A f(\mathbf{r}^N, \mathbf{p}^N)$, where $f(\mathbf{r}^N, \mathbf{p}^N)$ is the distribution function for the equilibrium ensemble, as may be seen from the equation (8) of PC. Calculating $\bar{A}(0)$ with this gives $\bar{A}(0) = \langle A^2 \rangle / \langle A \rangle$, which is equal to the right hand side of the equation (1), thus verifying the equation (1) for just this particular ensemble. But that does not make the result valid for an arbitrary non-equilibrium ensemble. If in the true spirit of linear response theory, one follows the section 8.5 of the book by Chandler⁵ and takes the non-equilibrium ensemble to be proportional to $e^{-\beta(H + \Delta H)}$, with H being the Hamiltonian, and $\Delta H = -fA$, where f is an applied field that couples to A , then one obtains

$$\bar{A}(0) = \langle A \rangle + \beta f \langle (A - \langle A \rangle)^2 \rangle. \quad (11)$$

As one can choose the coupling field f arbitrarily, it is clear that one does not have any inequality of form $\bar{A}(0) \geq \langle A \rangle$, as claimed by Pearson². As the inequality is not valid, one must clearly view the results obtained by Pearson with caution.

I thank the Alexander von Humboldt Foundation, Germany for supporting this work, by the gift of a computer system.

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Biobatteries to utilize bioenergy from fruit and vegetable wastes

Biomass and biowastes represent a large potential energy resource that is renewable on a sustained basis¹. Production of energy from biomass and biowaste resources became globally popular because of decreasing fossil energy supplies and increasing cost and demand for energy, a growing awareness of environmental impact associated with fossil fuel usage as well as surplus global biomass production and potential resource of biowaste produce². Several research studies^{3–7} have highlighted

production of solid, liquid and gaseous fuels from biomass and agricultural waste produce. In the recent past, generation of electricity from biowaste materials and/or municipal garbage was also made possible by converting the waste materials into biogas which in turn was used for the production of electricity based on the most advanced turbine technology².

Although Hwang *et al.*⁸ developed the concept of biocells, later led to their preparation using bacterial membranes

and platinum–silver electrodes to generate voltage in light⁹, attempts to produce electric power directly by electrochemical method from biowaste resources have been limited. Low power dry cells, which are widely used for calculators, wall clocks, video game toys, etc. are now becoming expensive and often non-available in time at village level. Therefore, we have attempted to prepare biobatteries utilizing commonly-available domestic biowaste materials and to use them in low voltage

appliances as alternatives to the conventional low power dry cells (batteries).

Ripe banana and orange peels or vegetable scrap, which are thrown as domestic waste (garbage) were collected and macerated into a fine paste without adding water. Pieces of copper and zinc sheets (0.5 mm thickness) were collected from metal scrap merchants. Copper and zinc plates measuring 10 cm² (4 cm × 2.5 cm) area were cut and selected as electrodes. Each electrode was connected with a short piece of insulated wire through a small hole made at one end of each electrodes. Electrodes were inserted into about 20 g of paste of fruit or vegetable waste taken in (30 ml) plastic vials. Similarly electrodes were inserted directly into

Table 1. Output of power generated in biobatteries prepared from different plant materials (fruits and vegetables). Measurements of power generated in each type of biobattery were repeated on batteries which were constructed using fresh samples of the same material and with the same set of electrodes. Data represent the mean \pm SD of at least five measurements made on different batteries

Material used	Power output of biobattery	
	Volts	mA
Brinjal	0.60 \pm 0.08	0.52 \pm 0.08
Cabbage	0.68 \pm 0.06	0.24 \pm 0.06
Carrot	0.60 \pm 0.09	0.21 \pm 0.05
Cattle dung (gobar)*	0.66 \pm 0.08	0.98 \pm 0.16
Lemon (ripened)	0.76 \pm 0.05	0.57 \pm 0.07
Orange (peels)	0.64 \pm 0.12	1.04 \pm 0.10
Banana fruit (peels)	0.74 \pm 0.05	1.33 \pm 0.12
Potato	0.62 \pm 0.05	0.72 \pm 0.05
Raddish	0.58 \pm 0.08	0.69 \pm 0.04
Sweet potato	0.56 \pm 0.08	0.92 \pm 0.06
Tomato (ripened)	0.62 \pm 0.12	1.15 \pm 0.16
Vegetable scrap**	0.60 \pm 0.10	0.96 \pm 0.08

*Represent livestock waste biomass.

**Represent vegetable waste biomass from kitchen.

Table 2. Performance and/or durability assessment of biobatteries prepared from peels of ripened banana fruit. Measurements of cumulative output of power generated from three biobatteries which were connected continuously in series to wall clock to make it work were made at weekly intervals over a period of four weeks. Data represent the mean \pm SD of three replicated measurements

Time of measurement (weeks)*	Power output	
	Volts	mA
0	1.97 \pm 0.15	1.36 \pm 0.12
1	1.92 \pm 0.11	1.27 \pm 0.09
2	1.85 \pm 0.09	1.24 \pm 0.15
3	1.80 \pm 0.10	1.20 \pm 0.08
4	1.69 \pm 0.12	1.13 \pm 0.09

*Weeks after preparation of biobatteries.

fresh and intact vegetables/fruits used in this study (Table 1) to make biobatteries. Electric potential generated in each battery was measured by multimeter on DC mode. Each biobattery was found to produce a low voltage power measuring 0.5–0.8 V and 0.2–1.4 mA (Table 1). Therefore a number of biobatteries were connected in series to increase the voltage output. Cumulative output of power generated in 2–4 biobatteries prepared with paste of ripened banana fruit peels was applied to wall clocks or calculators or video game toys through suitable connections using insulated electric wire and was found to make them function without using conventional low power dry cells. Life/durability of newly-constructed biobatteries was assessed by measuring output of power generated in biobatteries at regular time intervals over a period of four weeks and/or using them continuously for wall clocks or calculators. It was found that these batteries exhibited potential and were found functional over a substantial period of time (more than four weeks) without changing electrodes or electrolytic medium (Table 2). Further, it was found that performance of biobatteries could be improved by frequent cleaning of electrodes with water and without disturbing electrolytic medium. The

results showed that among the electrolytic media tested, the paste of ripened banana fruit peels with copper–zinc electrode combination was found effective for the performance of biobatteries. Studies on the evaluation of factors influencing the performance of biobatteries show that the size and quality of both electrodes and medium of electrolyte are important. Further, studies on chemical analysis of electrolyte viz. the paste of fruit and/or vegetable wastes used in biobatteries would be useful to understand the electrochemical mechanism(s) underlying the production of low voltage power in these biobatteries. However, further work on design and construction of biobatteries with different electrode combinations is needed to make them handy, durable and popular.

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REMI: A novel method for tagging genes

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Mutagenesis and gene cloning have traditionally been two separate tasks for the geneticist. But the realization that a transposon can be used both as a mutagen and also as a 'gene tag' now enables one to identify and isolate genes at one shot, particularly in systems such as bacteria, yeast, *Drosophila*, *Caenorhabditis* and maize.

But moving transposons around has not been possible in some systems like cellular slime moulds and filamentous fungi. The cloning of genes associated with aberrant phenotypes still remains an arduous task. However the work of Schiestl and Petes¹ has kindled a ray of hope in this direction. Much to their surprise, they found that the transformation frequencies increased considerably when the yeast *Saccharomyces cerevisiae* was transformed with a linearized plasmid together with the restriction enzyme used for linearization. In other words, the restriction enzyme stimulated the integration of the plasmid into cognate restriction sites in the genome.

The evidence suggested a 'simple' mechanism. The restriction enzyme enters the cell along with the linearized vector and cuts at the restriction sites in the genome. The host repair system does its job but at a low frequency, the sticky ends of the vector pair up with the compatible ends generated in the genome by the restriction enzyme. DNA ligase ties the loose ends up.

Encouraged by this finding, Kuspa and Loomis explored the possibility of restriction enzyme mediated integration (REMI) in the obstinate cellular slime mold, *Dictyostelium discoideum*. Much to their delight, they found that the efficiency of transformation increased more than 20-fold and the plasmid integrated more than 70% of the time at the appropriate restriction site².

This raised the prospect of using REMI as a mutagenesis-cum-gene tagging tool in *Dictyostelium discoideum*. The plan of action was simple. Transform the cells with a piece of sticky

DNA carrying a selectable marker in the presence of the appropriate restriction enzyme and select for the transformants. Among the set of transformants generated, hunt for the one with the desirable mutant phenotype. If one is lucky to get a mutant, then isolating the gene into which the vector has inserted is not difficult. Cut the genomic DNA with a rare restriction enzyme. The fragment from the digest carrying the inserted vector can be rescued in *E. coli* and reintroduced into the wild type strain to disrupt the cloned gene by homologous recombination. The high frequency of mutants among the transformants would confirm that the phenotype was indeed because of a mutation in the cloned gene.

Kuspa *et al.* and Adachi *et al.* have executed the plan successfully by cloning the genes involved in cell-cell interaction and cytokinesis in *Dictyostelium discoideum* respectively^{3,4}. REMI has been attempted in other systems, as well. Shi *et al.* have reported REMI enhanced transformation in the rice blast fungus *Magnaportha grisea*⁵. Success has also been reported in the maize pathogenic fungus *Cochleobolus heterostrophus*⁶. However, the presence of restriction enzyme seemed to have had no effect on transformation efficiency in *Neurospora crassa*⁷. There are no explanations as of now, why REMI works in some systems and not in others.

A new technique for physical mapping has been possible by using REMI in league with RFLP mapping. Kuspa and Loomis did REMI with a vector carrying a rare site Apa I within to generate a set of 147 transformants in *Dictyostelium discoideum*. An Apa I digest of the genomic DNA of the transformants yielded 2 fragments carrying the regions of the vector flanking the internal Apa I site. The probing of these 2 fragments with probes from the cloned genes enabled them to map the genes relative to the Apa I sites. Kuspa and Loomis published the detailed maps of

the six chromosomes of *Dictyostelium discoideum* using this technique, REMI-RFLP mapping^{8,9}. This method obviates the need to go in for other genetic data necessary for RFLP mapping.

REMI as a tool for insertional mutagenesis should enable one to identify genes which are not present in functionally redundant forms and not required for viability. The encouraging sign is that restriction enzymes have had no effect on the viability of the cells presumably because of an efficient host-repair system.

There is a point of doubt whether the integration is random¹ or non-random². Despite the possible non-random nature of insertions, the number of transformants could be increased by using different restriction enzymes. REMI also holds a lot of promise in physical mapping in creating chromosomal aberrations and inducing mitotic recombination.

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A new tool for plant gene isolation – Transposon traps

C. Santhosh Kumar and K. K. Narayanan

Transposable elements or transposons are segments of DNA present in many organisms that have a tendency to jump from place to place. They were first discovered in maize through the pioneering work of Barbara McClintock¹ much before the DNA structure was known. Similar genetic elements were subsequently discovered and characterized in bacteria, yeast, animals and other plants. Many such mobile elements have now been cloned and sequenced.

A typical transposable element is capable of autonomous transposition and is characterized by specific terminal sequences and an internal sequence which codes for a transposase. The transposase is essential for transposition. In plants, these transposable elements are organized into families. Each transposable element family, typically, consists of an autonomous member and several related non-autonomous or dependent members. All the members of a family have the same termini and create the same length of host sequence duplication at the site of insertion. The transposition event is regulated by the 'autonomous' family member which encodes the full length transposase protein that brings about transposition of all members of that family. Both insertion and excision of the dependent elements are contingent on the presence of a transcriptionally active autonomous element.

Transposons have long been used as a mutagen. Transposon tagging has now become a widely used tool for isolation of genes, both in pro- and eukaryotes. However, this approach, like the conventional mutagenesis approach, suffers from a major limitation. The target loci should have an obvious phenotype, if it has to lend itself to isolation by these approaches.

It is now well known that many genes in any organism, particularly eukaryotes, have no phenotype upon disruption. For example, in the yeast, *Saccharomyces cereviceae*, it is estimated that 60–70% of the genes have no obvious mutant phenotype^{2,3}. Further, genes which are spatially and/or temporally regulated, pose problems in mutagenesis screens. The recently developed approach of using transposon traps, a modification of the transposon tagging technique permits the easy identification and isolation of such genes.

The use of the modified transposon tagging or mutagenesis approach to enable the 'trapping' of genes or their regulatory sequences (enhancers) was pioneered in *Drosophila*⁴. The major modification in this new approach was the use of a synthetic transposon carrying a reporter gene that could confer expression patterns corresponding to the normal expression of the disrupted region. In plants, the potential of this technique was recently demonstrated in

Arabidopsis using the heterologous maize transposable system⁵.

The presence of autonomous and dependent transposable elements in plants offers an unique advantage. Stable transgenic lines, one carrying an immobile transposase source and the other, the dependent element can be produced and maintained. Transposition can be initiated by crossing the two. Further, stable transposants, or the line in which the transposon has moved, can be fixed in the segregating generations. The transposon trap constructs used in this technique, thus have two components. One, the terminal *cis*-acting sequences flanking a reporter gene or the mobile dependent element (Figure 1a and b) and two, the immobilized transposase source (Figure 1c). When separated, each component is stable. When brought together in a single line by crossing, the dependent element transposes to new sites with the help of the constitutively expressed, immobilized autonomous element. The expression of the reporter gene helps to monitor such movements. The genetic region of interest now flanks the dependent element and can be amplified by Inverse Polymerase Chain Reaction (IPCR).

The transposon trap elements can be designed to isolate either the gene itself (gene trap, Figure 1a) or its control element(s) (enhancer trap, Figure 1b). The gene traps are based on the proper integration of a mobile element carrying a reporter gene which lacks its own promoter sequences, into an actively expressed genetic locus. When the trap element integrates into an exon, in the proper reading frame and orientation the reporter gene is expressed. To ensure that the trap works if it gets inserted into an intron, appropriate splicing signals are provided immediately upstream of the reporter gene sequence. The enhancer trap construct carries a minimal (weak) promoter, upstream of the reporter gene. When this element gets inserted into the vicinity of an actively transcribed region, the reporter gene is activated by the neighbouring regula-

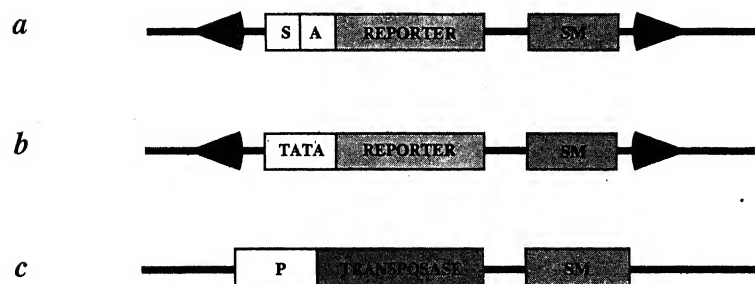


Figure 1. Transposable element constructs for trapping genetic regions. The solid arrows represent the conserved terminal sequences required for transposition and SM is the selectable marker. **a**, gene trap construct; S, conserved splicing signal; A, splice acceptor. **b**, enhancer trap construct; TATA, minimal promoter. **c**, immobilized transposase construct; P, strong promoter.

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tory sequences. The technique of enhancer trapping allows the expression pattern of a large number of genes to be visualized and screened rapidly.

The use of gene and enhancer trap elements can be an attractive alternative to other gene isolation techniques in plants. The generation of large number of transposon insertions by simply crossing a small number of transgenic parental lines and visual selection based on reporter gene expression make this method very efficient. It has now been established that the maize transposable

elements work in several heterologous hosts and this extends the application of this technique to species in which transposons are yet to be identified.

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Fishery genetics: An emerging discipline

B. K. Padhi and R. K. Mandal

Fishery genetics has grown rapidly in the last one and a half decades accumulating so much of knowledge and information of applied interest that it can be assigned the status of a discipline.

GENETICS has now acquired an important status in fishery science. There is growing interest on the subject as revealed by multifold increase in the number of research papers on cytogenetics, molecular, quantitative and biochemical genetics¹, suggesting the utility of applications of genetic principles and methods for improving the fish breeding programme, for documenting genetic diversity in natural populations² and developing proper management programmes to conserve genetic diversity³. In the present article the developments, scope and importance of the subject are discussed.

Fishery genetics – a definition

It is tempting to provide a comprehensive definition to this growing discipline. Since the subject concerns the applied aspects of fish culture and natural fishery, it appears more appropriate calling the subject 'fishery genetics' rather than 'fish genetics'.

Fishery genetics may be defined as the application of genetic principles and methods for increasing aquaculture productivity by genetically modifying fish stocks and for the management of fish populations to obtain maximum sustainable yield without affecting the genetic diversity.

Factors leading to the growth of the subject

The interest in fishery genetics grew primarily for two reasons. It was gradually realized that the anthropogenic factors such as over-exploitation of natural fishery from open waters, modification of aquatic environment by pollution load, development programmes like damming of rivers, etc. have led to a decline of wild populations and these have genetic and evolutionary consequences⁴. Also, the necessity was felt for improving aquaculture productivity by genetic improvement of fish stocks primarily to meet the increasing demand on fish proteins. The implications of each of these factors are discussed below briefly.

Over-exploitation and habitat modification

Effect on production trait

Evidence shows that traits such as growth rate and age of maturity of brood fish and feed conversion efficiency

are heritable and cultured populations of fish respond to selection for these characters⁵. It is now recognized that harvesting can exert an evolutionary selection pressure that may bring about genetical changes⁶. For example, most fishing gear selects a certain size of fish. Big hooks cannot catch small fish and wide meshes allow small individuals to wriggle through. There is a considerable variation in growth rates in fish of the same age group. Thus, it is expected that the fishing gear selects out fast growers in an age class. If growth rate is partly determined by the genetic factors, it is likely that prolonged inadvertent selection against fast growth will lead to selection of slow growers. Experiments with tilapia (*Oreochromis mossambica*) showed that harvesting has a negative selection pressure⁷. The other traits which harvesting can affect are the ability to escape net, body proportions, number of eggs per unit weight, etc.

Effective population size

Excessive mortality due to over-harvest or habitat modification and impairment of reproductive ability because of pollution load⁸ reduces the effective population size. Effective population size means the number of reproductively active individuals in a population. If the population size gets small, it results in inbreeding and genetic drift.

Effects of stock introduction and unwanted hybridization

The term 'introduction', in this context, means transfer of fish by man into waters outside of their native ranges. In other words, any intentional or accidental release of fish by human activity into the natural waters is considered introduction⁹. Introduction has ecological and genetic effects. The ecological effects of introduction include processes or mechanisms such as competition, predation and habitat alteration. The genetic effects of introduction leads to alteration of gene pools of indigenous species. The alteration may be direct as in the case of introgression (gene flow) or indirect such as reduction of effective population size, making natural population vulnerable to the effects of genetic drift. Intra-specific or inter-stock crossing leads to reduction of

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genetic variability between the populations (a homogenization effect)¹⁰. Therefore, there has been a growing interest in identifying F1 and F2 hybrid in nature and distinguishing them and also to document genetic introgression due to back-crossing of the F1 hybrid with parental species. Therefore, a number of research papers appeared, which dealt with introgressive hybridization primarily using isozymes as the tool¹¹ supplemented by mitochondrial DNA analysis. A few researchers used nuclear DNA RFLP¹².

Aquaculture

Genetic studies in aquaculture have witnessed two different approaches; firstly, to reveal the genetic impacts of using small and closed populations in commercial hatchery; secondly, to improve the genetic potential of fish stock by selective breeding, chromosomal engineering, gene manipulation, sex and fertility control. The first aspect primarily aims at reducing/avoiding the improper breeding practices, while the second aspect is to develop superior strains of fish.

In the early eighties, many research papers appeared which recorded low quality of fish seed produced due to improper breeding practices¹³. Around the same time the feasibility of quick methods, such as chromosome¹⁴ and gene¹⁵ manipulation to improve the genetic performance of fish came to limelight. Chromosome manipulation (by thermal, pressure and chemical shock) in fish was an interesting development. Because, unlike other vertebrates, fishes can tolerate change in ploidy level, and thus bypass long-term selective breeding programme, while trying to obtain superior variety.

Scope of applied genetic research in fishery science

At present there lies ample scope for applied genetic research in fishery science. Major areas needing proper research input for the conservation and productivity improvement of this renewable resource are as follows:

Stock identification

Identification and documentation of intra-specific genetic polymorphism occurring in different populations occupying different ecological zones within a species is of enormous practical significance. A number of research papers on this aspect are already available primarily from USA and other developed countries. However, this is a very virgin field in India and many other developing nations, where most of the fish biodiversity exist. This study is useful in selective breeding programme in hatchery, devising policy on the rehabili-

tation and conservation of a declining stock, biodiversity documentation, study of phylogeography and systematics and proper utilization of cryopreserved gametes¹⁶.

Stock/species introduction

Introduction is an useful approach to augment fish production by supplementing natural recruitment. However, introduction of exotic species/non-native stock for this purpose has been a matter of genetic concern. Many papers published recently pointed out that inter-stock crossing could lead to stock admixture and reduction of genetic biodiversity^{17,18}, which in turn would affect the future breeding programme.

Dowling *et al.*¹⁹ pointed out that introgressive hybridization was an evolutionary factor which could bring about genetic variability. On the contrary, many researchers viewed that introgression would cause genetic contamination² and decline of populations by bringing about the rearrangement in co-adapted gene complexes²⁰.

However, many relevant questions still remain; i) How much gene flow between or within the species is permissible? ii) Is the reduced population size after genetic interaction of inter-specific hybrid due to rearrangement of co-adapted genes or due to reduced fecundity (that is partial sterility) of the later generation hybrids? iii) How much threat does 'introduction' – as a factor, pose to the intra- and inter-specific genetic diversity? Further studies on measuring of gene flow among the populations and between the species would be of great importance.

Stock improvement

The remarkable developments in aquaculture are primarily due to availability of fish seed by artificial breeding and improved management practices such as supplementary feeding, use of clean water, eradication of pests and weeds, etc. Now that the technology of production and rearing has largely been mastered for many species of culturable fishes, it is reasonable to look for genetic techniques to increase aquaculture productivity. Genetic modification of the organism to increase the growth rate has been the primary goal of stock improvement programme to improve the aquaculture productivity. Enormous research efforts have already been put into this. The technical approaches have been selective breeding and hybridization, chromosome and gene manipulation, fertility and sex control. The practical benefit of genetic improvement programmes have already been manifested in Norwegian salmon industry, where accumulated selective breeding for 15 years showed improved productivity. Some scientists have

applied integrated approach (use of more than one technique) to develop better strains. For example, joint application of endocrine and cytogenetic approach to obtain monosex fish²¹ and production of tetraploid fish by chromosome manipulation and hybridizing it with the related diploid species to obtain triploid individuals with better characteristics.

In spite of methodological developments and so much of experience that is being recorded in the literature, quite a lot is yet to be done to fulfil the genetic goal set by the aquaculture industry for developing superior strains of fishes.

Monitoring genetic change

Breeding of a small-sized population, in nature or in hatchery, leads to loss of intra-population variability because of selection (intentional or unintentional), genetic drift and inbreeding. Reduction of genetic variability can be monitored in terms of reductions of heterozygosity by analysing the frequency of polymorphic loci or average number of alleles per locus. This would be useful in maintaining effective population size.

Indian scenario in fishery genetics research and education

In India, some research efforts were made on chromosome engineering²², transgenic fish production²³, sex control by endocrine and cytogenetic manipulation^{21,24}, cryopreservation of gametes²⁵, genetic stock identification²⁶ and hybrid identification²⁷. However, a lot more research efforts on fishery genetics are needed to generate knowledge, process and products of applied interest in aquaculture and capture fishery management.

Fishery genetics has not found its proper place in fishery education in the country. Fishery is taught as a special paper in M Sc Zoology courses in several Universities and there are about a dozen fishery colleges affiliated to agricultural/veterinary universities, offering courses on B FSc and M FSc. A preliminary survey revealed that fishery genetics is either not included in the syllabus or if some preliminary topics are included, they are not dealt with seriously. Although Indian Council of Agricultural Research has set up the National Bureau of Fish Genetic Resources in Lucknow more than a decade ago, there is a general apathy towards fishery genetics among the academicians and policy makers associated with fishery education and research.

Concluding remarks

Annual world landings of aquatic resources have increased more than four-fold; from 21.9 million tonnes

per year between 1952 and 99.5 million tonnes in 1989 (ref. 28). The larger share of this production came from the capture fishery sector, which has been over-exploited, leading to decline of fish biodiversity. An analysis revealed that 20% (1800 species) of the world's freshwater species are severely threatened²⁸. On the other hand, aquaculture provides greater scope for increasing fish production and productivity. Thus, fishery genetics as a growing discipline will have an important role to play in future for conservation and management of natural fishery and increasing the aquaculture productivity.

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Protein–DNA and protein–protein interactions in λ -repressor/operator system: A review

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λ -repressor is a two-domain protein. The N-terminal domain interacts with the DNA, while the C-terminal domain is responsible for all protein–protein interactions. Functioning of the repressor in the natural context implied that the two domains should have flexibility. Denaturation and fluorescence anisotropy studies, however, demonstrate that the situation is more complex. It is likely that in the free repressor the two domains interact with each other and are not completely free to rotate. The binding of operator to N-terminal domain, however, causes a conformational change in the C-terminal domain. This conformational change leads to change in protein–protein interaction. One of the major changes that is seen, is that of the role of the C-terminal tail region in protein–protein interaction. It is hypothesized that the role of the conformational change is to couple the specific operator binding information to the protein–protein interaction, which may lead to increased specificity at the required sites.

REGULATION of information flow from DNA to RNA to protein is at the center of all biological phenomena. This regulation, both when following an internal programme and in response to an external stimulus, is primarily at the level of transcription. The regulation of transcription is a very complex process in eukaryotes and is not well understood. Even the basal transcription in yeast may take tens of protein factors¹. Such complex systems are difficult to study from structural points of view with the present level of technology. Prokaryotic and phage regulatory systems are often much simpler and at the same time incorporate many of the regulatory features of the higher eukaryotes^{2,3}. Thus, they are ideally suited for investigations by structural and biophysical techniques.

Many of the prokaryotic regulatory systems have been studied in fair details by genetic and molecular biological techniques. They include *Lac*, *Gal*, *Trp*, *deo* and *Ara* operon of *E. coli*⁷ and lytic-lysogenic regulatory system of bacteriophage λ ^{8–10}. Protein components of these and several other prokaryotic regulatory systems are well characterized and nature of the regulatory events have been described. Crystal and NMR structures of some of these proteins and some fragments have also been eluci-

dated^{11–14}. Thus, several of these systems are poised for further investigations of the regulatory events in quantitative terms and at atomic levels.

My laboratory has focused attention on the lytic-lysogenic switching system of bacteriophage λ . Extensive genetic and biochemical work from the early '70s by Mark Ptashne and coworkers has established the components and the basic nature of the system. The switching system consists of three contiguous 17 base pair sequences known as the right operator sites, O_{R1} , O_{R2} and O_{R3} , and two proteins, λ -repressor and *cro*. This collection of operator sites, known as the right operator or O_R , also contains two promoters, P_{RM} and P_R (Figure 1). The structural gene of the λ -repressor is under the control of the promoter P_{RM} and the structural gene of the *cro* is under the control of the promoter P_R .

The *cro* protein is a homodimer of a polypeptide chain of 66 amino acids and binds to the three operator sites, in the order of decreasing affinity, $O_{R3} > O_{R2} > O_{R1}$ (ref. 15). The *cro* protein functions as a down regulator of transcription¹⁶. In contrast, the λ -repressor is a multifunctional protein that acts as both *up* and *down* regulator depending on the context^{17,18}. In addition, λ -repressor binds to the three operator sites, co-operatively, in the order of decreasing affinity $O_{R1} > O_{R2} > O_{R3}$ (ref. 19). This co-operative binding is vital for the functioning of the genetic switch, since, otherwise normal non-cooperative mutants lose their ability to lysogenize completely. In the lysogenic state, two repressor molecules occupy O_{R1} and O_{R2} , and activate the promoter P_{RM} and repressing the promoter P_R , thus shutting off *cro* synthesis and continuing λ -repressor synthesis. The continuing repressor synthesis is required to replenish the dilution losses due to cell division and growth. No other gene is transcribed in this state and the steady-state is maintained. Following DNA damage, the *recA* protein cleaves the repressor, destroying the co-operative binding and weakening the affinity for the operator sites. This throws the switch, shutting off the repressor synthesis and initiating *cro* synthesis, resulting in entry into the lytic pathway (Figure 2).

It is clear from the above description of the operation of the switch, that the λ -repressor plays the central role in switching the phage from lysogenic to the lytic mode

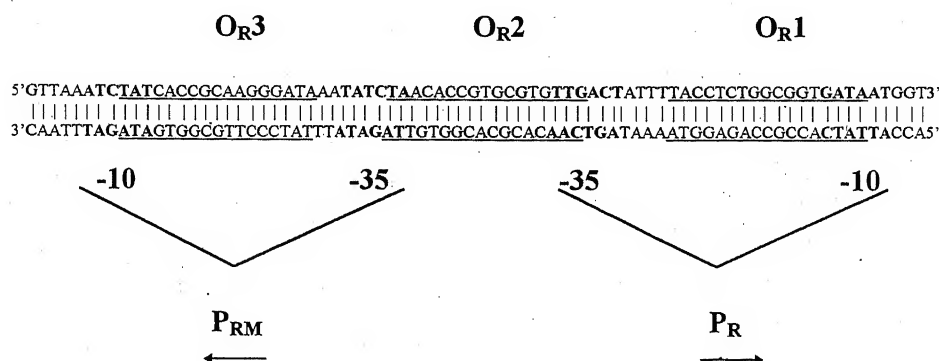


Figure 1. DNA sequence of right operator region of bacteriophage λ .

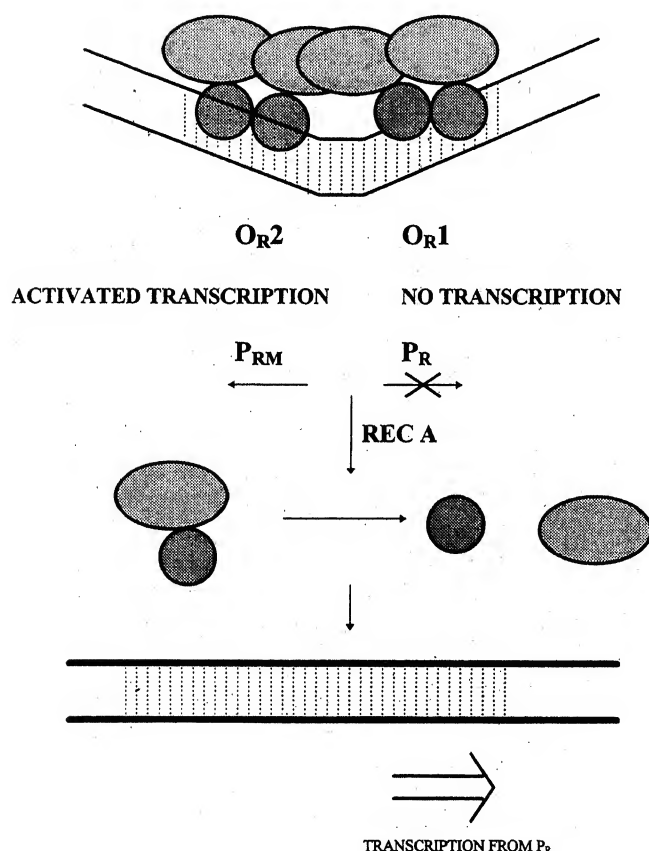


Figure 2. Depiction of $OR1-OR2/\lambda$ -repressor complex in lysogenic state and subsequent induction to lytic pathway. The upper half of the figure represents two repressor molecules bound to the operator sites $OR1-OR2$ and preventing transcription from P_R but activating transcription from P_{RM} . The middle part of the figure depicts the cleavage of the repressor by RecA and the bottom part represents the unbound form of DNA in which transcription from P_R takes place.

of growth. λ -repressor monomer consists of a single polypeptide chain of 236 amino acids. The monomers associate to form dimers, tetramers, octamers and at

very high protein concentrations, higher order structures^{20,21}. The chain folds into two domains, 1–92 and 132–236 with a hinge region in between the two domains²⁰. The N-terminal domain contains the helix–turn–helix motif, commonly found in many prokaryotic DNA-binding proteins, and has been shown to bind operator DNA²². The C-terminal domain is largely responsible for dimer and higher order aggregate formation as well as protein–protein contact needed for co-operative binding to multiple operator sites²³. Very little is known about the structure of the hinge region or its function.

A problem of great magnitude, such as regulation of gene expression and genetic switching, requires study of many systems. Understanding of their diversity and underlying unity is essential to unravel the complexity of the problem. One or a few emerge as the paradigm. *Lac* and λ regulatory systems have perhaps emerged as the best characterized systems in terms of genetics, biochemistry and structure. Thus, the stage was set for quantitative biophysical studies to understand the regulation in quantitative terms. Seminal work by Gary Ackers and co-workers^{24–26} in the eighties has established the basic physicochemical parameters of the system, such as binding affinities, cooperative interaction energies, etc. The task now is to elucidate conformational and dynamical aspects of the system, so that finally it can be understood at the residue or the atomic level.

My laboratory has undertaken a long term programme to investigate the biophysical aspects of functioning of the λ genetic switch, in general and the interactions of λ -repressor, the most important component of the switch, in particular. The interactions of λ -repressor with the operator sites have been studied in detail by Gary Ackers and his co-workers, using quantitative footprinting^{24,25}. They measured the net co-operative interaction energies, which turn out to be small, around 2–3 kcal/mole. This net co-operative interaction energy consists of at least the following elements: (i) The

proximity effect of bringing the two adjacent molecules to one another. This effect, in analogy to the chelate effect, should be entropic in origin. (ii) The protein-protein interaction energy while bound to the two operator sites. (iii) The DNA binding energy. (iv) The protein distortion energy. The last two effects oppose the first two. The decomposition of the net co-operative interaction energy to its constituent parts is essential if one has to understand co-operatively in molecular and atomic terms. Thus, we have focused our attention on these individual effects, concentrating on protein-protein interaction, DNA binding and DNA-induced conformational change.

Domain-domain interaction in λ -repressor

It was generally believed that the two domains of the repressor may be free to rotate, with the hinge acting as a connecting flexible region. This putative flexibility was thought to be important in the functioning of the repressor since, the repressor can co-operatively bind to two operator sites separated by variable number of base pairs. The crystal structure of the isolated N-terminal domain as well as its complex with the O_L1 , is known^{12,22}. Very little information on the C-terminal domain and hinge structure is, however, available. NMR studies of the whole repressor molecules suggested motional freedom of the N-terminal domain due to the observed line narrowing²⁷. The experiments were, however, done at very high protein concentrations (3–5 mM) where λ -repressor is known to oligomerize to higher order structures, which may have properties different from those of the free dimer. Equilibrium denaturation of λ -repressor by urea has been studied using fluorescence and circular dichroism spectroscopy²⁸. λ -repressor contains three tryptophan residues, 129, 142, 230 and three cysteine residues, 180, 215 and 219. The λ -repressor denatures in three distinct phases: in the first phase, which is centered around 2.0 M, there is a significant red shift of tryptophan fluorescence, but little change in far-UV circular dichroism spectrum. The second phase, centered around 3.5 M urea, is characterized by large decrease of the CD spectral intensity, but no change in the tryptophan fluorescence spectrum. The third phase occurs at very high urea concentrations, above 8 M urea. Fluorescence polarization studies indicate that the repressor remains as a dimer and the dissociation into monomers occurs around 7.5 M urea. As mentioned above the λ -repressor contains three cysteine residues, all of which are situated in the C-terminal domain. The attempted reaction of the cysteines with DTNB indicated that the cysteines were unreactive in the native state. Exhaustive protease digestion, however, produced near-full reactivity, suggesting inaccessibility as the cause of inertness. This lack of reactivity is pre-

served even at 6 M urea, suggesting that the first two phases of denaturation do not involve total denaturation of the C-terminal domain. One sulfhydryl group becomes available for reaction at urea concentrations higher than 8 M. The intact repressor can be cleaved by papain into 1–92 and 132–236 fragments, which can be isolated²⁰. Denaturation of the isolated N-terminal domain coincides with the second phase of the denaturation, suggesting that the second phase is the denaturation of the N-terminal domain. This also agrees with the lack of tryptophan residues in this domain (no change in tryptophan fluorescence) and high alpha helical content (large change in CD signal). The nature of the first transition thus could be partial denaturation of the C-terminal domain or the hinge region or both and hence, denaturation of the isolated 132–236 fragment was studied. This fragment still shows the first phase of the denaturation, only the mid-point shifts to around 1.0 M urea concentration. This suggests that the first phase of the denaturation involves denaturation of the part of the C-terminal domain. Under controlled proteolysis condition, the repressor can be cleaved by papain to produce the fragment 93–236 (ref. 20). This fragment has also been purified. The equilibrium denaturation shows that the first denaturation phase is centered around 1.4 M urea. These data suggest that the presence of the hinge region and the N-terminal domain confers additional stability to the part of the C-terminal domain which undergoes denaturation in low urea concentrations. Since the fragment 132–236 preserves the first transition involving tryptophan residues, W142 or W230 must be responsible for the red shift. N-Bromosuccinimide modification studies suggest that W230 is highly red shifted (λ_{\max} around 345 nm). Since the first transition involves red shift of one tryptophan residue having λ_{\max} of less than 340 nm, it suggests that W142 may be the tryptophan involved in the first transition. Thus, it may be concluded that the N-terminal domain, the hinge and the N-terminal part of the C-terminal domain may interact with each other in the native repressor and the two domains may not be completely isolated. The interaction energy, however, may be small (approximately 1 kcal/mole; obtained from the extrapolation to zero urea concentrations), suggesting that the freedom of internal rotation may be gained by sacrifice of only around 1 kcal/mole energy.

The concept of interacting domains enunciated above received strong backing from fluorescence polarization studies. As mentioned above, the repressor lacked reactive cysteine groups to be tagged with appropriate fluorophores. The protein may be labelled with dansyl chloride without the loss of biological activity²⁹. Rotational correlation time, under conditions known to favour the dimer, was measured from Perrin plot. The result obtained was consistent with a globular dimeric protein of molecular weight of 52 kDa. If the domains

were completely free to rotate with respect to each other, then this would have led to smaller values of the rotational correlation time.

Self-assembly of λ -repressor

The preparation of dansyl labelled repressor with full biological activity produced a unique opportunity to study protein association in λ -repressor. λ -repressor is known to be in a monomeric state at concentrations below nanomolar level. It dimerizes with an association constant of about 10^8 M^{-1} and remains predominantly dimeric till micromolar concentrations^{26,30}. Early qualitative work suggested that beyond $1 \mu\text{M}$, λ -repressor formed tetramer, octamer and eventually higher order structure²¹. The self-assembly was not known in quantitative terms. A quantitative measurement of the self-assembly is important to elucidate the nature of biologically important protein-protein interaction.

Using fluorescence anisotropy measurement, the assembly profile of λ -repressor from dimer to tetramer was determined. Under the solution conditions used (i.e. in the absence of divalent cations), the tetramer to octamer transitions was largely decoupled from the dimer to tetramer transition. The temperature dependence of the assembly profile showed the dimer to tetramer assembly to be highly temperature dependent and enthalpy driven with a ΔH of around -34 kcal/mole . The binding of the single operator site fragment O_R1 caused weakening of protein-protein interaction around ambient temperature. At about the same time other laboratories had reported a concerted transition from dimer to octamer approximately the same concentration range, using ultracentrifugation³¹. Their solution conditions, however, included millimolar concentrations of Ca^{++} and Mg^{++} . Controversy about the correct model of self-assembly lingered on for some more years, until recently, when two studies from different laboratories resolved the issue. Gary Ackers and co-workers have shown that the original concerted dimer to octamer model was based on inaccurate analysis of the ultracentrifuge data and the revised data showed much lower degree of coupling between the two transitions under the conditions previously reported to favour concerted dimer to octamer assembly (G. A. Ackers personal communication). Studies in our laboratory suggested that millimolar divalent cation concentrations promote significant coupling of the two transitions and in the absence of divalent cations only a small degree of coupling is seen³². Whether the effects of divalent cations on protein association have corresponding effect on protein-protein interaction responsible for binding co-operativity, is unknown at the present moment.

Operator-induced conformational change

The arguments presented above suggest that the N- and the C-terminal domains in λ -repressor may not be free to rotate. That raises an apparent dilemma. The various operator site pairs are separated by a variable number of base pairs (four through seven) and the repressor binds to these pairs of operator sites with co-operative interaction energies of similar magnitude. When the operator sites were separated by a much longer stretch of DNA on an artificial construct, the repressor still bound to these operator sites co-operatively³³. Such insensitivity to the length of the intervening DNA suggests considerable flexibility in the C-terminal domain, since the N-terminal domain remains anchored to the operator site. It is possible that co-operative contact occurs between two operator sites bound dimers at the cost of N-terminal domain-C-terminal domain interaction. The other possibility is that binding of repressor to the operator site may lead to a global conformational change, leading to facilitated freedom of movement of the C-terminal domain. The possibility that binding of the operator site to the λ -repressor produces a global conformational change was investigated, using intrinsic tryptophan fluorescence and an environment-sensitive fluorescence probe, bis-ANS³⁴. All the tryptophans of λ -repressor are situated away from the N-terminal domain and hence the DNA binding site. The binding of an oligonucleotide containing an isolated operator site, O_R1 , leads to significant quenching of tryptophan fluorescence and a modest shift of emission maximum. Since all the tryptophans are situated away from the DNA binding site, the operator-induced change is likely to be transmitted to the C-terminal domain. Bis-ANS is an environment-sensitive probe with affinity for the apolar sites of proteins. Bis-ANS binds to the C-terminal domain of λ -repressor. Binding of the oligonucleotide containing O_R1 operator site causes fluorescence enhancement of the bound bis-ANS, indicating a global conformational change occurring upon binding of the oligonucleotide containing the operator site. Whether such a global conformational change leads to increased freedom of movement of the C-terminal domain, remains to be seen.

Role of C-terminal tail in protein association

During studies of protein association, it was observed that a shift of emission maximum of tryptophan fluorescence takes place during protein association at the concentration range that is known to promote dimer to tetramer association under the conditions used. This suggested that one of the three tryptophans may be at or near the protein-protein contact site and its identification may lead to localization of part of the dimer-dimer

contact site. Acrylamide quenching experiments showed that accessibility of at least one tryptophan is significantly different in the dimeric and tetrameric state³⁵. This tryptophan also shifts its emission maximum from very red, 345 nm in the dimer to very blue 334 nm in the tetramer. N-Bromosuccinimide oxidation leaves one of the tryptophans unmodified in the tetrameric state, but not in the dimeric state. Peptide mapping, amino acid analysis and sequencing suggest that this tryptophan is W230. Based on this information we created a site-directed mutant F235C for attachment of fluorescence probe to the tail region of the protein. This sulfhydryl residue can be selectively labelled with an environment-sensitive probe, acrylodan. Acrylodan 235C-labelled protein showed significant shift of emission maximum and fluorescence enhancement upon association from dimer to tetramer. Thus, we conclude that the whole C-terminal tail region of the protein may be involved in the dimer-dimer association.

Interestingly, many non-cooperative mutants have been isolated by several groups and some of them are mapped in the C-terminal tail region of the protein^{23,36}. But most of the mutants are mapped in the other parts of the C-terminal domain. Several of the mutant proteins have been studied in respect to their association properties and DNA-binding abilities. The only well-characterized tail mutation is S228N. Although this was originally characterized as a non-cooperative mutant¹⁰, recent studies have shown that it is defective in monomer-dimer association and not co-operativity defective as was originally suggested^{37,38}. Sedimentation velocity ultracentrifugation studies have shown that S228N does not associate beyond the dimer stage, i.e. aggregation defective. This study pointed towards the fact that the role of the tail in co-operative contact and free protein association may be different. Since there are two excellent markers, W230 and C235, for the tail region, we decided to explore this interesting issue further.

A double operator site, O_{R1}-O_{R2}, containing oligonucleotide was synthesized. Binding of λ -repressor to this oligonucleotide leads to protein-protein contact, while the repressor dimers remain bound to the two adjacent operator sites. This complex was studied using acrylamide quenching as a tool³⁵. The acrylamide quenching pattern of the O_{R1}-O_{R2}/repressor complex is very similar to the dimer, indicating no change of environment of W230. Acrylodan-labelled protein at cysteine 235 also shows different behaviour. The AC235-repressor shows dramatic quenching of AC fluorescence upon single operator titration and similar effect is seen with double operator titration. This is in contrast to the fluorescence enhancement seen in the free repressor undergoing tetramer association. The thermodynamics of the protein association is also dramatically different in free protein tetramer formation and co-operative contact. Whereas the former is strongly enthalpy driven, the

latter is iso-enthalpic and entropy driven. The difference in thermodynamics and the role of the C-terminal tail may be due to spatial constraint imposed upon the protein-protein association by the proximity of the two adjacent operator sites and rigidity of the DNA, or it may be due to a change in nature of the protein-protein interface, induced by operator binding. The protein-protein association in single operator site bound dimer is, like the co-operative interaction energy, iso-enthalpic and entropy driven. The behaviour of W230 is also unlike the free tetramer association in the operator bound dimer association. We may conclude from these studies that free dimer association is significantly different from co-operative dimer-dimer contacts and this difference is a result of the global conformational change induced by the binding of the operator.

Binding of λ -repressor also induces a conformational change in the double operator fragment O_{R1}-O_{R2} as seen by difference circular dichroism spectra. This distortion is not seen in a control oligonucleotide of the same composition where the two operators have been placed on different sides of the DNA by insertion of half turn of the DNA. This suggests that co-operative interaction of the λ -repressor is responsible for the distortion of the DNA and the distortion probably originates from the intervening stretch between O_{R1} and O_{R2}. This result is consistent with DNase I hypersensitive band seen in intervening sequence by quantitative footprint experiments³⁹.

Significance of the operator-induced conformational change

Based on the fact that the operator binding causes a global conformational change in the repressor, which ultimately leads to change of protein-protein interaction and DNA distortion induced by protein-protein co-operative contact, we propose that the λ -repressor/O_{R1}-O_{R2} complex is a strongly coupled system, in which, operator binding, DNA distortion and protein-protein interaction are strongly coupled. This can be understood in greater detail from the thermodynamic cycle depicted in Figure 3. The sum of intrinsic interaction energies (including entropic gain due to proximity effect) is not fully expressed, and the observed binding energy is less.

$$\Delta G_{ob} = \Delta G_{dis} + \Delta G_{bin} + \Delta G_{int}.$$

ΔG_{ob} is lower than $\Delta G_{int} + \Delta G_{bin}$. The difference is used to pay for the distortion of the DNA and protein. Clearly the central question is, what is the role of DNA and protein distortion in the system?

One possible answer is that the distortions are needed to bring the system in right geometric disposition. We feel that this is a red herring, and the real significance lies elsewhere. It was previously observed that

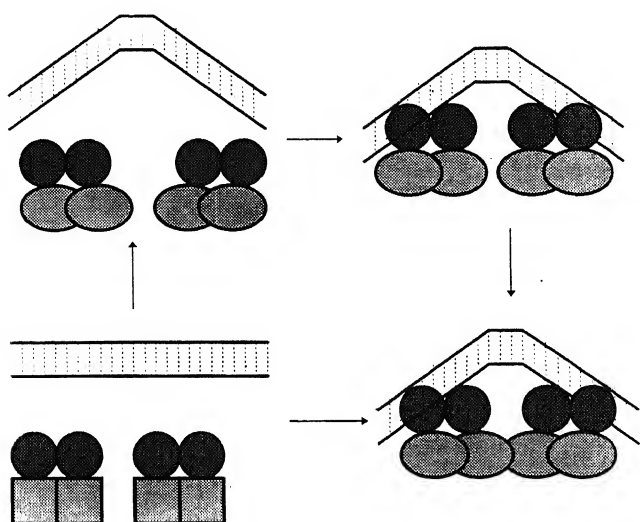


Figure 3. Decomposition of net co-operative interaction energy into individual interaction energies using binding cycle. The transformation from unbound DNA and unbound protein (lower left corner) to co-operatively bound complex (lower right hand corner) releases ΔG_{oh} . The free repressor C-terminal domain is represented by a square, whereas the operator-bound conformation is represented by an ellipse. The transformation from lower left to upper left, represents distortion of DNA and protein and is represented by ΔG_{dis} . The transformation from upper left to upper right represents binding energy of the distorted states, ΔG_{bin} . The transformation from upper right to lower right represents the protein-protein interaction energy while the repressor molecules are bound to the two adjacent operators, ΔG_{int} .

non-specific DNA sequences do not induce the conformational change induced by specific operator sequence. This suggests that the conformational change may be a mechanism to couple the correct recognition of sequence to appropriate protein-protein interaction and subsequent complex formation. This may prevent formation of a complex at inappropriate sequences.

Is there any other possible role for the conformational change and strong couple? In the classical model of *down regulation*, the repressor down regulates transcription by excluding the polymerase from the promoter. Recent work has suggested that this may not be the case⁴⁰. A number of studies have shown that polymerase and repressor can coexist on the DNA^{40,41}. Adhya and coworkers have hypothesized that like positive regulation, the negative regulation also takes place through repressor-polymerase protein-protein contact². In a collaborative unpublished work⁴² we have shown that under defined *in vitro* conditions, in the *gal* system, induction of transcription occurs at repressor concentrations much above the dissociation constant of the operator-repressor complex. This suggests that obligatory steric occlusion may not be the mechanism of *down regulation* in all cases and the repressor-polymerase contact should be considered as a serious possibility for the mechanism. If such a possibility turns out to be true, I speculate that another role for the operator-induced

conformational change may be to present the correct interface for interaction with RNA polymerase, causing *down regulation*. In any non-specifically bound situation, such interface is not available for interaction with the RNA polymerase, thus decreasing the potential for causing harm elsewhere.

In summary, the regulatory system of bacteriophage λ has been investigated in detail from genetic, biochemical and to a moderate extent from structural points of view. Further structural investigations remain to be done. Quantitative biophysical parameters have been established from Gary Ackers' laboratory. We have shown the importance of conformational change and dynamics in the functioning of such a regulatory system. The challenge is now to understand the control and switching of transcription at the atomic level and in quantitative terms.

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REVIEW ARTICLE

Oxidative phenol coupling: A key step for the biomimetic synthesis of many important natural products

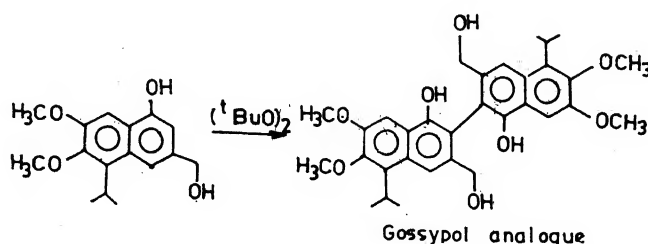
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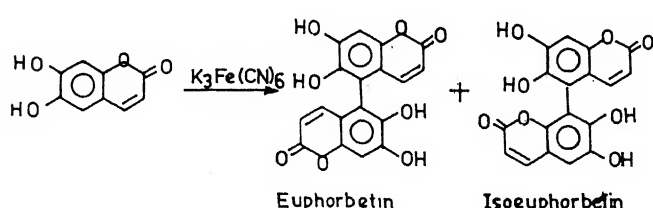
We present here the oxidative phenol coupling (OPC) reaction utilizing various reagents and leading to biomimetic synthesis of many natural polyphenolics.

OXIDATIVE phenol coupling (OPC) reaction incarnates a 'C–C' or 'C–O' bond between phenolic moieties through oxidation as delineated in all the schemes described here. OPC thus becomes a fundamental method for the synthesis of hydroxylated biaryls¹. The reaction has previously been of little significance and synthetic value because it could not be controlled to the desired step. Unwanted side products were also formed if the aromatic substrate had several sterically or electronically comparable positions. But, later, it gained tremendous attention with the discovery of a prodigious number of natural products such as lignans,² xanthenes³ and a wide variety of tetrahydroisoquinoline alkaloids⁴, which had been assumed^{5–7} and later confirmed to be biosynthesized involving OPC of appropriate precursors. This reaction thus gained a new dimension by

providing an excellent laboratory method for mimicking certain biosynthetic steps. This has initiated extensive studies on the reaction on a wide variety of phenolic compounds, leading to the successful biomimetic synthesis of many natural products, viz. gossypol analogue,⁸ a well-known male antifertility agent (Scheme 1); euphorbetin and isoeuphorbetin⁹, two coumarin dimers (Scheme 2); several alkaloids¹⁰; dimers



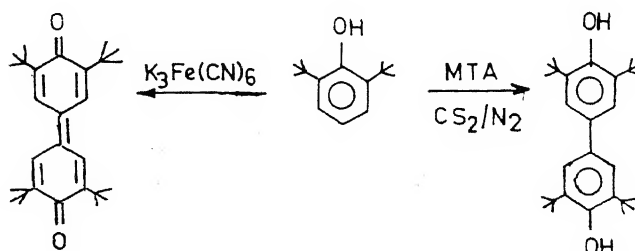
Scheme 1.



Scheme 2.

of hydroxylated phenanthrenes and dihydro phenanthrenes¹¹, constituents of high altitude Himalayan orchids; polyhydroxy flavones¹²; lignans¹³; xanthenes¹⁴ and many more compounds. The importance of the reaction is also clear in the light of different medicinal and biological utility of many coupled products. Among these properties are antitumour-anticancer activity, hormonal characteristics, colouring properties, and drug activity. Often attempts to duplicate a natural product lead to the formation of another isomer¹⁵ not occurring in nature. With time, extensive diversification of the OPC reaction has grown with respect to variations in the phenolic and related substrates as well as through the availability of newer oxidizing agents and optimization of the reaction conditions. Achievement of regiospecificity and stopping the reaction at the desired stage is now possible in many cases.

Reagents of both inorganic and organic origin such as $FeCl_3$ (ref. 16), $K_3Fe(CN)_6$ (ref. 17), $AgOH$ (ref. 18), $K_2Cr_2O_7$ (ref. 19), $KMnO_4$ and K_2MnO_4 (ref. 20), iodobenzene diacetate²¹, active MnO_2 (ref. 22), manganese tris acetylacetonate (MTA)²³, tert-butyl peroxide, isoamyl nitrite²⁴, $O_2/NaOH$ (ref. 18), RuO_2 (ref. 25) and enzymes²⁶ have been used for this purpose. Irradiation by ultrasound in the presence of an oxidizing agent²⁷ has also been an efficient method for product formation. Solid-supported reagents such as Ag_2CO_3 on celite²⁸, $FeCl_3$ bound to SiO_2 (ref. 29), phosphomolybdic acid adsorbed on silica gel¹¹ also proved to be efficient reagents for the phenol coupling process. These special types of reagents are responsible for easier handling, separation procedures and higher efficiency. Anodic oxidations and oxidation via photochemical radical generation³⁰ are also viable methods for the OPC reaction. However, anodic oxidation may lead to solvent incorporation³¹, and with MTA, abnormal products may result³². Use of $AgNO_3$ (ref. 33) and chromyl chloride³⁴ may cause nitro and chloride group insertion. Sometimes the OPC reaction causes formation of phenyl ethers³⁵. Sometimes an attack at the benzylic position competes with the desired coupling process. Also, sometimes, OPC reaction results in quinones³⁶ or coupled quinone³⁷. A general theory has not been set up governing the formation of product; but variation of reagent and condi-



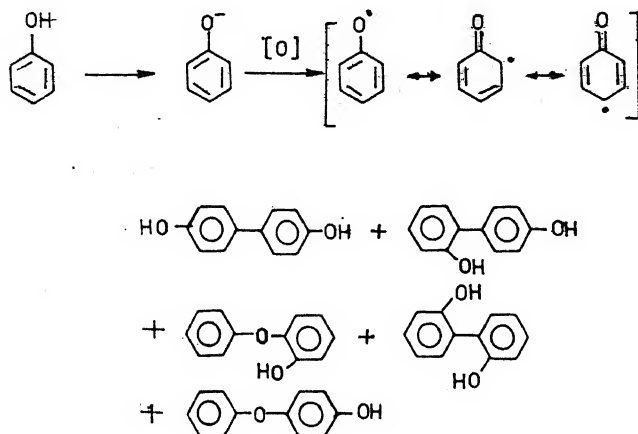
Scheme 3.

tion may lead to different products (Scheme 3) and sometimes isolated examples appear in the literature showing excellent yield. Reaction with another class of reagents including VOF_3 , $VOCl_3$, VCl_4 , thallium(III) tris trifluoroacetate (TTFA) may result in a 'non-phenolic' dehydrodimerization of electron-rich aromatic compounds³⁸.

An examination of the standard reduction potential (E^0) values for most of the reagents suggests that a reagent with lower E^0 value would behave in a milder way and cause less polymerization. This has been accomplished using Ag-gelatin complex³⁹, a weaker oxidizing agent than $AgOH$ or $AgNO_3$. Based on the E^0 values, uranyl(VI), rhodium(III), platinum(IV), and Hg(II) complexes would be expected to react similarly as reagents in the OPC reaction. One such observation on the OPC reaction is the coupling of 2,7-dihydroxy naphthalene by HgO (ref. 40).

Radical-initiated OPC to form polycyclic dimers and trimers has been observed using natural montmorillonite and smectic clays⁴¹ with favoured regio- and stereochemical control. The influence of steric, electronic and also hydrophobic character of the substrates has been documented with biocatalyst⁴² in nonaqueous solvent. Even now, newer inorganic reagents^{43,44} are being considered for successful coupling reaction. The underlying but obligatory condition is to impose milder conditions⁴⁵ to warrant better coupling instead of quinone formation. Thus macrocyclic chelates⁴⁶ and catalyst⁴⁷ have been employed. To achieve success, alteration of reaction conditions, purging of oxidizing gas have also been looked into. Stepwise coupling⁴⁸ has recently widened the horizon of OPC reaction to the greatest extent in relation to selectivity, complex-induced proximity effect, regiochemical control and percent yield conversion point of view.

Mechanistically it is believed that the first step in OPC is the generation of a phenoxy radical by the transfer of an electron to the oxidant as evidenced from the e.s.r. studies. The generated phenoxy radical then is thought to undergo a mostly homolytic coupling (Scheme 4). In extreme cases an ionic mechanism may also operate. While this mechanistic aspect has been



enlightened, more extensive work is necessary for understanding the precise mechanism of the entire reaction and there is no warranty that all phenol couplings proceed by one and the same mechanism¹.

In view of the many-fold applications of OPC reaction, one may think aloud about constructing bi- and polyaryls in a viable and efficient route. Apart from mimicking nature, this coupling process may be a passport for entering into the world of macrocycles⁴⁸. It is quite probable that this synthetic method may be accomplished by various means and is amply described in literature.

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A symposium on 'High Energy Physics in the 21st Century' was held on 11 November 1995 at the Indira Gandhi Centre for Atomic Research, Kalpakkam as a part of the 61st Annual Meeting of the Indian Academy of Sciences, 10–12 November 1995 at Madras. The four themes of the symposium were: *The Standard Model of High Energy Physics, Theoretical Scenarios for 10^3 GeV to 10^{19} GeV, Hints of New Physics from Cosmology, Astrophysics and Nonaccelerator Experiments, and New Ideas on Acceleration to Planckian Energies.* Talks bearing on these themes were given by D. P. Roy, Romesh Kaul, Ramanath Cowsik and Abhijit Sen respectively. Three of these talks along with an introductory overview (by GR) and a final summary (by RR) which were also given in the Symposium are included in the following collection.

G. Rajasekaran
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High Energy Physics in the 21st century – An overview

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At the beginning of the 20th century, the quest for the understanding of the atom topped the agenda of fundamental physics. This quest successively led to the unravelling of the atomic nucleus and then to the so-called 'elementary' particles – proton, neutron, pion, electron, etc. Particle Physics or High Energy Physics (HEP) made rapid strides in the latter part of this century, especially in the 60s and 70s. These developments culminated in the construction of the gauge theory of quarks and leptons. This theory which goes by the rather prosaic name of 'The Standard Model (SM) of High Energy Physics' was constructed almost in its final form by 1973 and since then it has passed with flying colours all the experimental tests performed in the next 20 years. So, at the end of the century, we now have a highly successful theory which is valid, down to a distance scale of about 10^{-16} cm (8 orders of magnitude smaller than the atomic scale). This theory can now be regarded as *the basis of all of physics except gravity*. This success story is described in the article on page 111 by D. P. Roy.

1900 A.D. —————→ 2000 A.D.
Atoms → Nuclei → Particles → Gauge theory of quarks
and leptons
——————————→
 10^{-8} cm 10^{-16} cm

The SM is a theory for all that is known in HEP, namely the weak, electromagnetic and strong interactions of the quarks and leptons. However, this is not the end of the story. There are too many loopholes and unsolved problems within SM: Higgs and symmetry breaking, QCD and confinement, neutrinos, CP and its violation, etc. The solution of these problems may already take us beyond SM.

However, the biggest loophole in SM is the omission of gravitation, the most important force of nature. Hence, it is now recognized that *Quantum Gravity (QG) is the next frontier of HEP*, and that *the true fundamental scale of physics is the Planck energy 10^{19} GeV*, which is the scale of QG.

In quantum mechanics, there is an inverse relationship between the length scale and the energy needed to probe that scale. A few characteristic length and energy scales are given below:

Landmark	Length	Energy
Nuclear physics	10^{-13} cm	200 MeV
Standard model	2×10^{-16} cm	100 GeV
↓	↓	↓
Quantum gravity	2×10^{-33} cm	10^{19} GeV

One can see the vastness of the domain one has to cover before QG is incorporated into physics. In their attempts to probe this domain of $10^2 - 10^{19}$ GeV, theoretical physicists have invented many ideas such as supersymmetry, supergravity, hidden dimensions, etc and based on these ideas, they have constructed many beautiful theories, the best among them being the superstring theory, which may turn out to be the correct theory of QG. These 'Theoretical scenarios for 10^3 GeV to 10^{19} GeV' is the topic of Romesh Kaul's article (page 116).

But, physics is not theory alone. Even beautiful theories have to be confronted with experiments and either confirmed or thrown out. Here we encounter a serious crisis facing HEP. In the next 10–15 years, new accelerator facilities with higher energies such as the Large Hadron Collider ($\sim 10^4$ GeV) or the Linear Electron Collider will be built and so the prospects for HEP in the immediate future appear to be bright. Beyond that period, the accelerator route seems to be closed because known acceleration methods cannot take us beyond about 10^5 GeV. It is here that one turns to 'Hints of new physics from cosmology, astrophysics and nonaccelerator experiments'. This topic was covered by Ramanath Cowsik in the Symposium. (Unfortunately, his talk could not be included in the present collection.)

Very important hints about neutrinos, dark matter, etc. have come from Astrophysics and Cosmology. Nonaccelerator experiments on proton decay, neutrino masses, double beta decay and 5th force are important since they provide us with indirect windows on superhigh energy scales.

In spite of the importance of astroparticle physics and nonaccelerator experiments, these must be regarded as only our first and preliminary attack on the unknown frontier. *These are only hints!* Physicists cannot remain satisfied with hints and indirect attacks on the superhigh energy frontier. *So, what do we do?*

As already mentioned, the outlook is bleak, because known acceleration methods cannot take us far.

To sum up the situation, there are many interesting fundamental theories taking us to the Planck scale and even beyond, but unless the experimental barrier is crossed, these will remain only as metaphysical theories.

It follows that either, *new ideas of acceleration have to be discovered* or, *there will be an end to HEP by about 2010 A.D.*

It is obvious what route physicists must follow. Hence, we have the very important article – 'New ideas on acceleration to Planckian energies' by Abhijit Sen (page 121). Some of the ideas being pursued are laser beat-wave method, plasma wake field accelerator, laser-driven grating linac, inverse free electron laser, inverse Cerenkov acceleration, etc. What we need are a hundred crazy ideas. Maybe, one of them will work. Lawrence's discovery of the cyclotron principle is not the end of the road. Here lies an opportunity of discovery our country should not miss.

A task force for new methods of particle-acceleration

It is high time that we form a small group of people as the task force whose aim shall be to do research and discover new methods of acceleration. This should be a multidisciplinary team including laser, plasma and accelerator specialists. It must include particle-detector specialists also, since the success of this venture will ultimately depend on our mastery of not only new methods of particle-acceleration but also new methods of particle-detection. One or two theorists with bright ideas may play a useful role in this group.

What is contemplated is *not* a new Institute or a new Department in any existing institute, but rather a think-tank of young people in existing institutions who are prepared to commit a significant part of the next 25 years of their careers to this visionary dream of creating a Planckian accelerator in the laboratory. These must be people with their eyes and minds turned on the heavens but with their feet placed securely on the earth and their hands actively engaged in the laboratory. They must be visionaries prepared to gamble on crazy ideas, but at the same time, having the capacity to test the ideas in down-to-earth laboratories *in this country*.

One must guard against big expenditure of money; the emphasis must rather be on investing on ideas. Clearly, what is expressed above is only the sketch of a proposal. Much more thought is required, to carry it forward to the practical stage. Hopefully, if the seed is planted now, it may grow and fructify in the next 25 years.

Finally, we must face the question: why should we do High Energy Physics? The answer can be given in the form of the following questions.

Is quantum mechanics for ever? Is relativity for ever?

Classical physics failed to explain the stability of the atom and thus quantum mechanics was born. Quantum gravity suggests that quantum mechanics may fail to explain the stability of space-time and so a new form of mechanics may be necessary. Such new discoveries may come only through the study of deeper regions of space-time. From this point of view, the onward march to shorter distances (higher energies) assumes a great significance. *Ultimately, the real justification for pushing the frontier of HEP to higher energies is the expectation that we will reach the boundaries of validity of our present view of the physical universe – based on our present conceptual framework of space-time (relativity) and our present understanding of dynamics (quantum mechanics) and that the new discoveries will take us beyond those boundaries.*

The standard model of fundamental particles

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This is an overview of our current understanding of the fundamental particles and their interactions, which is commonly called the Standard Model. The major developments in this field are outlined along with the major issues that remain to be settled. Important Indian contributions to these developments are underlined.

OUR concept of fundamental particles has undergone two revolutionary changes during this century. The first was the Rutherford scattering experiment of 1911 bombarding α particles on gold atom; while most of them passed through straight, occasionally, a few were found to scatter off at a large angle. This showed that the atom is not a solid compact object, but is largely hollow with a solid compact nucleus (made up of protons and neutrons) surrounded by the tiny revolving electrons. The second was the electron-proton scattering experiment of 1968 at Stanford, which was essentially a repeat of the Rutherford scattering type experiment, but at a much higher energy. The result was also very similar as illustrated in Figure 1. It was again clear from the scattering pattern that the proton is not a solid compact object itself, but is largely hollow with three solid compact cores called quarks. Indeed we know now from many such experiments, that all the nuclear particles (proton, neutron and mesons) are made up of quarks, i.e. they are quark atoms.

The reason it takes a much higher beam energy to probe the structure of the nuclear particles is their small size relative to the atom, about 1 fm (10^{-15} m) against 1 Å (10^{-10} m). It follows from the famous Uncertainty Principle that the uncertainties in distance and energy are related by

$$\Delta x \Delta E > \hbar c \sim 0.2 \text{ GeV} \cdot \text{fm}, \quad (1)$$

i.e. probing a distance scale $x \ll 1$ fm requires a beam energy $E \gg 1$ GeV, where a Giga electron volt ($\text{GeV} = 10^9 \text{ eV}$) is the energy acquired by an electron on passing through a billion volts. It is this multi-billion volt accelerator technology that is responsible for the half a century gap between the two experiments. The Stanford experiment got the Nobel Prize in 1990.

In particle physics one normally uses the so-called natural units

$$\hbar = c = 1, \text{ i.e. } m = mc^2. \quad (2)$$

That means the mass of a particle is same as its rest mass energy. The GeV is the most commonly used unit of mass, energy and momentum. The mass of proton $m_p \simeq 1 \text{ GeV}$.

Basic constituents of matter

According to our present understanding the basic constituents of matter are a dozen of spin-1/2 particles (fermions) along with their antiparticles. These are the 6 leptons (electron, muon, tau and their associated neutrinos) and the 6 quarks (up, down, strange, charm, bottom and top). Each can be organized into 3 pairs, in increasing order of mass (Table 1). Members of each pair differ by 1 unit of electric charge as shown in the last column, i.e., charge 0 and -1 for the neutrinos and the charged leptons and $2/3$ and $-1/3$ for the upper and lower quarks. This is relevant for their weak interaction. Apart from the electric charge the quarks possess a new kind of charge called colour charge. This is relevant for their strong interaction, which binds them together inside the nuclear particles (hadrons).

Basic interactions

There are 4 basic interactions among these particles – strong, electromagnetic, weak and gravitational. Apart

Table 1. Basic constituents of matter

Leptons	ν_e	ν_μ	ν_τ	0
	e	μ	τ	-1
Quarks	u	c	t	$2/3$
	d	s	b	$-1/3$

ν , neutrino; u , up quark; d , down quark; c , charm quark; t , top quark; s , strange quark; b , bottom quark.

Table 2. Basic interactions

Interaction	Strong	EM	Weak
Carrier	g	γ	W^\pm & Z^0
		$U(1)$	$SU(2)$
Gauge group	$SU(3)$	$SU(2) \times U(1)$	

g , gluon; γ , photon; W , charged weak boson; Z , neutral weak boson.

SPECIAL SECTION – HIGH ENERGY PHYSICS

from gravity, which is too weak to have any perceptible effect in particle physics, the other three are all gauge interactions. They are all mediated by spin-1 (vector) particles called gauge bosons, whose interactions are completely specified by the corresponding gauge groups (Table 2).

The three basic interactions are illustrated by Feynman diagrams shown in Figure 2. They are simple space-time pictures, where the arrows indicate the direction of time. It may be noted here that a particle line is equivalent to the corresponding antiparticle with the direction of the time arrow reversed. Thus the same Feynman diagram represents the scattering process

$$(a) \, qq \rightarrow qq, \quad (b) \, q\bar{q} \rightarrow q\bar{q}, \quad (c) \, d\nu_e \rightarrow u\bar{e}, \quad (3)$$

as well as the corresponding annihilation process

$$(a) \, q\bar{q} \rightarrow q\bar{q}, \quad (b) \, \ell\bar{\ell} \rightarrow q\bar{q}, \quad (c) \, d\bar{u} \rightarrow e^-\bar{\nu}_e, \quad (4)$$

where the bar denotes the antiparticle. The last diagram also represents the decay process

$$\mu^- \rightarrow \nu_\mu e^- \bar{\nu}_e \text{ or } d \rightarrow u e^- \bar{\nu}_e, \quad (5)$$

the latter being responsible for neutron decay. The rate of the scattering, annihilation or decay is simply given by the square of the corresponding Feynman amplitude given below each diagram, where Q^2 denotes the 4-momentum square transferred.

The quarks interact strongly by the exchange of a massless vector particle called gluon, whose couplings are proportional to their colour charge C (Figure 2a). This is analogous to the electromagnetic interaction between quarks and charged leptons by the exchange of the massless photon, whose couplings are proportional to their electric charge e (Figure 2b). The constant of proportionality for the strong interaction is called α_s in analogy with the fine structure constant α ($\simeq 1/137$) for the electromagnetic interaction. And in analogy with Quantum Electro-Dynamics the theory of strong interaction is called Quantum Chromo-Dynamics (QCD). There is an important distinction between the two interactions, however, which follows from the non-abelian nature of the strong interaction gauge group $SU(3)$. Unlike the photon which does not carry electric charge, the

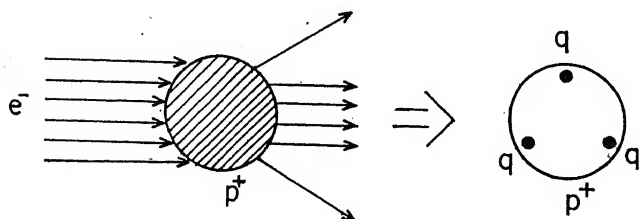


Figure 1. The Stanford electron-proton scattering experiment.

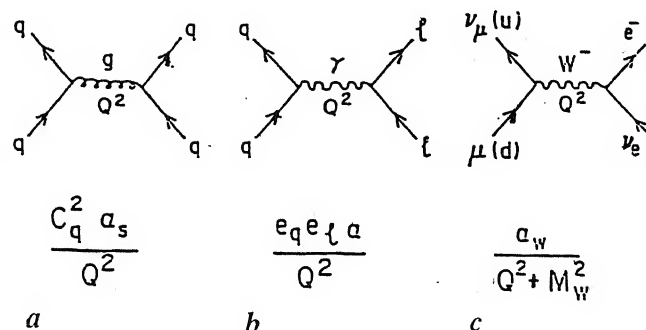


Figure 2. Feynman diagrams for (a) strong, (b) electromagnetic and (c) weak processes.

gluon carries a colour charge and hence has self-interaction. This is responsible for the most distinctive property of strong interaction called confinement, i.e. the quarks and gluons are perpetually confined inside the nuclear particles (hadrons); they cannot be pulled apart! The weak interaction between the quarks and leptons is mediated by massive vector particles called W^\pm and Z^0 bosons. The charged W boson couples to the above pairs of quarks and leptons with a universal coupling α_w (Figure 2c), since they all belong to the doublet representation of the weak gauge group $SU(2)$, i.e. they carry the same gauge charge. This is called the charged current weak interaction. The neutral Z boson couples to each quark and lepton, mediating the neutral current weak interaction, e.g.

$$q\nu \xrightarrow{Z} q\nu. \quad (6)$$

Electro-weak unification

The weak and the electromagnetic interactions have been successfully unified in terms of a $SU(2) \times U(1)$ gauge theory, for which Glashow, Salam and Weinberg were awarded the Nobel Prize in 1979. Being a product group it contains two independent gauge couplings, whose relative magnitude is defined in terms of a parameter called the weak mixing angle θ_w . The weak couplings α_w and α_z are related to the electromagnetic coupling α in terms of this parameter. Following the discovery of the neutral current weak interaction¹, the parameter θ_w was estimated² from the relative rates of the charged and neutral current weak interactions (3c) and (6). The present value of this parameter is

$$\sin^2 \theta_w \simeq 0.23. \quad (7)$$

Thus

$$\alpha_w = \alpha / \sin^2 \theta_w \simeq 4\alpha \simeq 1/30, \quad (8)$$

i.e. the weak coupling is in fact stronger than the electromagnetic. The rate of the weak process (Figure 2c) is weaker than the electromagnetic (Figure 2b) due to the

extra suppression factor coming from the large W mass. Note that this is only a low-energy phenomenon, occurring at $Q^2 \ll M_W^2$. The two rates are predicted to become comparable at $Q^2 \geq M_W^2$, as indeed they do. In other words the electro-weak unification takes place at the energy scale of the W boson mass. One can also predict the W boson mass from the observed rate of a low energy weak process like μ decay (Figure 2 c). One gets

$$M_W \simeq 80 \text{ GeV and } M_Z = M_W / \cos \theta_W \simeq 91 \text{ GeV,} \quad (9)$$

i.e. they are about 100 times heavier than the proton.

Discovery of the fundamental particles

The list of fundamental particles consists of the above-mentioned quarks, leptons and gauge bosons. The up and down quarks are the constituents of proton $p(uud)$ and neutron $n(udd)$. So together with the electron they constitute all the visible matter around us. The heavier quarks and leptons are unstable and hence not freely occurring in nature. But one can produce them in the laboratory experiments or detect them in the cosmic rays. The muon and the strange quark were discovered in cosmic ray experiments in the late forties, the latter in the form of the K -meson ($s\bar{d}$). The neutrinos are massless and stable, but hard to detect because of their weak interaction with matter. The ν_e was discovered in an atomic reactor experiment in the mid-fifties and awarded Nobel Prize this year (1995), while the ν_μ was detected at the Brookhaven proton accelerator in 1962 and awarded Nobel Prize in 1988. The first cosmic ray observation of neutrino (ν_μ) was made in the Kolar Gold Mine experiment in 1965 (ref. 3).

The remaining constituents of matter as well as the carriers of the basic forces have all been discovered during the last two decades, thanks mainly to the electron-positron and proton-antiproton colliders. In fact, over half of these discoveries were made during the seventies. The charm quark and the tau lepton were discovered at the Stanford e^+e^- collider in 1973 and 1975 respectively. The former got the Nobel Prize in 1976 and the latter got it this year (1995). The bottom quark was discovered at the Fermilab proton accelerator in 1977; but most of its detailed studies have been done at the e^+e^- colliders. The basic production mechanism is the electromagnetic annihilation process of (4b), where the kinetic energy of the colliding e^+e^- pair is converted into the rest mass energy of the produced particles ($m_c \simeq m_\tau \simeq 2 \text{ GeV}$, $m_b \simeq 5 \text{ GeV}$), (see Figure 3). At first the produced particles were recognized from the nature of their decay products. But it has been possible now to track them before their decay. The gluon was discovered at the Hamburg e^+e^- collider in 1979 in the form of 3-jet events. The basic production process is again the electromag-

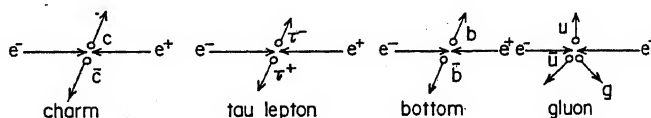


Figure 3. The discoveries of charm, tau, bottom and gluon.

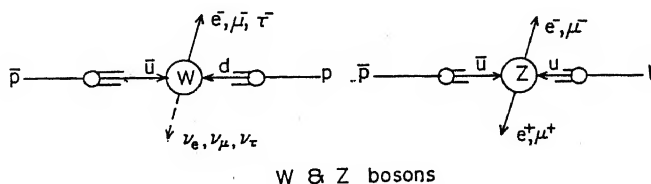


Figure 4. The discovery of W and Z bosons.

netic annihilation of e^+e^- into a quark-antiquark pair as in (4b), but followed by the QCD process of gluon radiation from one of the outgoing quarks.

One may ask how do these quarks and gluon come out of the confinement domain of $\sim 1 \text{ fm}$? The answer is that in quantum mechanics there can be many quarks and gluons floating in the vacuum, since their energy-momenta are taken care of by the Uncertainty Principle. Each of the produced particles picks up some extra quarks and gluons from the vacuum to come out as a colourless cluster of hadrons (mostly mesons). This results in an energetic jet of hadrons, carrying the energy and momentum of the produced particle. One expects only a small amount of momentum smearing, $\Delta p \sim 0.2 \text{ GeV}$, from the Uncertainty Principle (1).

The production of the massive W and Z bosons requires highly energetic beams, which was first achieved at the CERN $\bar{p}p$ collider (the $\bar{p}p$ collider has a higher energy reach than the e^+e^- machine, while the latter is better suited for detailed investigations). These particles were discovered there in 1983, which got the Nobel Prize the following year.

The underlying production mechanisms are the quark-antiquark annihilation process (4c) for W and the analogous neutral current process for Z (see Figure 4). Although the W and Z bosons decay instantly, they leave unambiguous imprints in their decay products as illustrated in the figure. One can easily infer about the production of W and Z bosons and measure their masses from these imprints. More recently millions of Z bosons have been produced at the Large Electron-Positron (LEP) collider at CERN resulting in a detailed study of the Z boson properties. The second phase of LEP, currently in progress, will produce W^+W^- pairs and make a detailed study of the W boson properties. An Indian group has been participating in

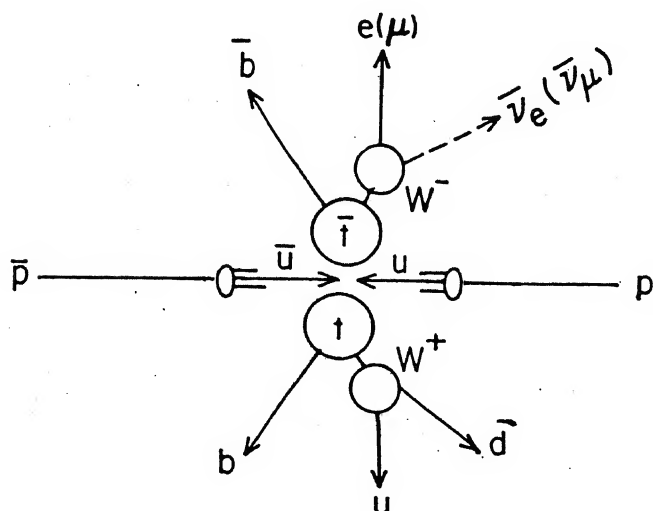


Figure 5. The discovery of the top quark.

one of the LEP experiments called L3, where it has made significant contributions to hardware, software as well as the data analysis. In particular, this group has been the main contributor to the line shape analysis of the Z boson signal⁴.

The last and the heaviest of these fundamental particles, the top quark, has been discovered this year (1995) at the Tevatron $\bar{p}p$ collider at Fermilab^{5,6}. Its mass is about 200 times larger than the proton mass, and, hence, it decays instantly like the W and Z bosons. But the characteristics of its decay products are far more complex. Figure 5 illustrates the production and decay of a top quark pair at the $\bar{p}p$ collider. The underlying mechanism for $\bar{t}t$ production is the QCD annihilation process of (4a), where the kinetic energy of the colliding $u\bar{u}$ is converted into the restmass energy of the $\bar{t}t$ pair. The decay mechanism is similar to that of (5).

One looks at the leptonic decay of one of the top quarks to avoid the QCD background from the scattering of stable quarks and gluons. The huge energy release in the decay of the massive top quarks implies that (i) the decay lepton (e or μ) comes out wide apart (isolated) from the other decay products and (ii) all the decay quark jets are hard, i.e. they carry large transverse-momenta. The first criterion helps to distinguish it from the b and c quark backgrounds and the second from the W boson background. The lepton isolation and jet hardness criteria for the top quark signature were first suggested in refs. 7 and 8 respectively. They have been widely used in the top quark search and its ultimate detection in the CDF⁵ and D0⁶ experiments at the Tevatron collider. Three Indian teams from Bombay, Chandigarh and Delhi are participating in the D0 experiment, where they have made significant contributions to the hardware and data analysis.

It is clear from the above discussion that particle physics has come a long way in the last 25–30 years. But the journey is far from over yet. There are several issues in strong and more importantly in weak interaction physics, which remain to be settled.

Confinement

We do not have a complete understanding of confinement yet, although there has been impressive progress in this direction in recent years. It has come in particular from the lattice gauge theory approach using large scale computer simulations, where Indian theorists have made notable contributions. A crucial prediction of this theory is the deconfinement of quarks and gluons into a quark-gluon plasma (QGP) in some extreme conditions, which may be achieved in relativistic heavy ion collision. Consequently, there is an extensive programme to search for QGP signal in the present and proposed heavy ion accelerators at CERN and Brookhaven. Experimental groups from Bhubaneswar, Calcutta, Chandigarh, Jaipur, Jammu and BARC (Bombay) are actively participating in this programme.

Mass problem: Higgs mechanism

The most serious problem of the Standard Model arises from the fact that the weak gauge bosons, W and Z, are massive. The gauge boson mass terms break the gauge symmetry of the Lagrangian, required for a renormalizable theory. The latter is essential for the cancellation of the infinities occurring in the theory. So the question is how to give mass to the weak gauge bosons without breaking the gauge symmetry of the Lagrangian? The clue is provided by the fact that spin-0 (scalar) particle masses are not constrained by any symmetry. This can be exploited to give mass to the gauge bosons through back door, i.e. they acquire mass by swallowing massive scalar particles. This is achieved via the Higgs mechanism of spontaneous symmetry breaking, which leaves the symmetry of the Lagrangian and hence the renormalizability of the theory intact. However it predicts at least one remaining scalar particle called Higgs boson in the mass range of W and Z bosons, which is yet to be detected.

Hierarchy problem: supersymmetry

Solving the mass problem via the Higgs mechanism leads to the so-called hierarchy problem, i.e. how to control the Higgs boson mass in the desired range of W and Z masses. This is because in the absence of a protecting symmetry the scalar masses have divergent quantum corrections, making them infinitely heavy. By

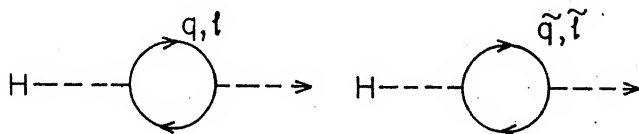


Figure 6. Divergent quantum correction to the Higgs boson mass coming from quark and lepton pairs (left) and its cancelling contribution coming from their superpartners (right).

far the most promising solution to this problem is provided by supersymmetry (SUSY), which is a basic symmetry between fermions and bosons, i.e. all fermions have bosonic superpartners and vice versa. It ensures cancellation⁹ between the divergent quantum corrections coming from the exchange of the standard particles and their superpartners as illustrated in Figure 6. In the process, however, one predicts a host of new particles, scalar partners of quarks and leptons as well as fermionic partners of gauge and Higgs bosons, to occur in the mass range of W and Z bosons. The two sets of particles are distinguished by a multiplicative quantum number called R -parity, which is $+1$ for all the standard particles and -1 for their superpartners. Conservation of R -parity implies that (i) the superparticles are produced in pair and (ii) the lightest superparticle (LSP) resulting from their decay is stable. The LSP is also required to have no colour or electric charge. Consequently, it is expected to interact weakly with matter and escape detection like the neutrino. This results in an apparent momentum imbalance (missing-momentum), which serves as a powerful signature for superparticle production. The first such signature for superparticle production at $\bar{p}p$ colliders was formulated by Reya and Roy¹⁰.

The Higgs and superparticles are the minimal set of missing pieces, required to complete the current picture of particle physics. It may not be the ultimate theory; but at least it will be a complete and self-consistent theory. As such the search for these particles are the prime physics goals of the present and proposed high energy colliders. In particular, the large hadron collider (LHC), scheduled to be completed in 2005 at CERN, will make it possible to carry the Higgs and superparticle searches right up to their predicted mass limits. An Indian team is actively participating in the R & D of one of the proposed experiments (CMS) at LHC, while several theorists are engaged in devising optimal search strategies for these particles at LHC using computer simulations. The observation of these particles will complete the current picture of particle physics in the way outlined above, while their nonobservation at LHC will provide important clues to an alternative route.

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Theoretical scenarios for 10^3 GeV to 10^{19} GeV

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Basic dogmas of particle physics are reviewed. Some of their implications beyond the standard model are explored. Higgs sector of the standard model of electroweak interactions is the weakest link in the model. Elementary Higgs field makes the model 'unnatural' beyond about 10^3 GeV. Supersymmetry provides the most attractive framework wherein this problem can be addressed. This new symmetry, relating fermions and bosons, is expected to be operative at about 10^3 GeV. In addition, grand unification of the fundamental interactions can be studied consistently only within a supersymmetric formulation. Inclusion of gravity with other interactions leads to supergravity theories, which should emerge as a low energy description of a more fundamental theory, the string-theory. Supersymmetry again is an essential feature of such a theory. Quantum gravity, with its characteristic scale of 10^{19} GeV, may well be described by a superstring theory.

TODAY, particle physics involves six quarks and six leptons as the fundamental constituents of all matter. It was only last year that the sixth quark, the 'top', was discovered. The quarks come in three-somes, the three colours. The quarks and leptons experience four types of basic forces, electromagnetic, weak nuclear, strong nuclear and gravitational. The electromagnetic and weak forces are described by the model of Glashow, Salam and Weinberg¹⁻³, while the strong interactions experienced by the quarks are believed to be governed by Quantum Chromodynamics (QCD). These together form the Standard Model of particle physics. Einstein's general theory of relativity provides an excellent description of the gravitational forces at large distances. However, short distance picture of gravity, where quantum effects become relevant is still puzzling. In this sense, quantum gravity is the least understood of all the basic forces of Nature.

The present theoretical understanding of the fundamental constituents and the forces experienced by them is governed by four guiding principles or dogmas: (i) Gauge principle, (ii) Renormalizability (or finiteness for theories with gravity), (iii) Naturalness principle, and (iv) Unification of the fundamental forces of Nature. We shall review these ideas in the following.

Gauge dogma

Every fundamental force has an underlying gauge principle. The oldest known gauge principle is that of electromagnetic interaction. The physical consequence of this principle is that this force is carried by a spin one massless vector gauge quantum, the photon, with an underlying gauge group $U(1)$. Two other fundamental interactions, the weak and strong nuclear forces are also governed by gauge theories. In the successful unified picture of electromagnetic and weak interactions due to Glashow, Salam and Weinberg¹⁻³, we have a gauge theory of four spin 1 gauge quanta. One of these is massless and is identified with the photon. Other three are massive, two charged W_{μ}^{\pm} and one neutral Z_{μ}^0 . These mediate weak nuclear forces. The underlying gauge group of this theory is $SU_L(2) \times U_Y(1)$ which is spontaneously broken down to an electromagnetic $U(1)$. Discovery of these gauge bosons, W^{\pm} and Z^0 in 1983, a major event in the history of science, confirmed the now thirty-year-old electroweak theory.

The strong nuclear force which holds the quarks together in a proton or neutron is described in terms of eight spin one gauge bosons, the gluons, corresponding to a gauge theory based on colour group $SU_C(3)$.

The gravitational interaction, usually thought of in terms of geometric properties of space-time, is also compatible with the gauge dogma^{4,5}. This force is mediated by a postulated spin two massless field, the graviton. Experimental discovery of this gauge particle is an outstanding problem. Whereas non-gravitational forces are described by the gauge theories of internal symmetry groups, the gravitational interaction is governed by the gauge theory of space-time symmetries. In this sense gravity is somewhat different.

Principle of renormalizability

All the known non-gravitational fundamental forces of nature are described by renormalizable quantum gauge field theories. These theories combine the building blocks of modern physics, special theory of relativity, quantum and gauge principles. Quantum field theories are generically plagued with infinities. In renormalizable field theories, there is a systematic way of absorbing

these infinities into redefinition of the fields and parameters, leaving behind only finite values for them. Unfortunately, this is not true for quantum theory of gravity. The root of the problem is that, unlike other quantum gauge field theories, description of gravity necessarily, involves a dimensionful coupling constant, Newton's gravitational constant. This makes one-loop or higher divergences in this theory to have a functional form other than that of the quantum action. Therefore, the usual prescription of absorbing these infinities back into the original quantum action by rescaling the parameters and fields is not available. In fact the gravitational quantum field theories may in general never be perturbatively renormalizable.

There are only two extreme options for the gravitational field theories: either the infinities of the S -matrix cannot be removed at all, or the infinities on their own are altogether absent. Thus, an acceptable quantum theory of gravity should be finite on its own – there should be some inner mechanism such that the divergences cancel. Some symmetry may provide such a mechanism. Obviously, finiteness of the S -matrix is a stronger constraint than renormalizability. But, it appears that quantum gravity has to choose a more miraculous way of existing other than the soft option of renormalizability that other interactions adopt.

Naturalness dogma

According to this dogma, existence of a small parameter in Nature cannot be an accident, there must be an associated symmetry. This is best formulated as follows:

't Hooft's doctrine of naturalness: At any energy scale μ , a set of physical parameters, $\alpha_i(\mu)$ may be small, if and only if in the limit $\alpha_i(\mu) \rightarrow 0$, the system has an enhanced symmetry⁶.

The weakly broken symmetry ensures that the smallness of the parameter is stable against perturbative influences. An example of a perfectly natural theory is quantum electrodynamics. The electromagnetic coupling α , the electron mass m_e , the muon mass m_μ , etc. can all be independently small. The smallness of m_e (or m_μ) is protected by the fact that, in the limit $m_e \rightarrow 0$ (or $m_\mu \rightarrow 0$), we have an additional symmetry corresponding to the separate conservation of the left- and right-handed electron-like leptons. All corrections to the electron mass due to the quantum fluctuations are small, proportional to m_e itself. Also, $\alpha \rightarrow 0$ enhances symmetry; it implies no interaction; hence the particle number of each type is conserved.

On the other hand, field theories with elementary scalar fields are not natural. There is no approximate sym-

metry that protects the smallness of the scalar mass. Electroweak theory has an elementary scalar field in its Higgs sector. This provides masses to the weak gauge bosons W^\pm , Z^0 through the so-called Higgs mechanism breaking the electroweak gauge group $SU(2) \times U(1)$ to the electromagnetic $U(1)$ at about 100 GeV. This ensures the renormalizability of such a theory of massive non-abelian gauge bosons. Though Higgs particle is central to our present understanding of structure of matter, yet it has stayed completely elusive. Experimental discovery of this particle is a major outstanding problem of present times. That is why Leon Lederman has nicknamed it as 'the God Particle'⁷. Being an essential part of the standard model, at the same time it renders the electroweak theory 'unnatural'. That is, the 100 GeV scale of this theory is no longer stable under perturbative quantum corrections. For definiteness, the correction to Higgs mass m_H due to quantum fluctuations of a size characterized by a scale Λ is

$$\delta m_H^2 \propto \alpha \Lambda^2,$$

say, at one loop level. Corrections to the masses of weak gauge bosons W^\pm and Z^0 are also of the same order. This is because these gauge bosons acquire masses through spontaneous symmetry breaking whose scale is controlled by the Higgs mass. Thus, if $m_H \sim 100$ GeV, and $\alpha \sim \frac{1}{100}$, and if we wish that the Higgs mass does not receive large corrections, $\delta m_H \sim m_H$, we have:

$$\Lambda^2 \sim \frac{\delta m_H^2}{\alpha} = \frac{(100 \text{ GeV})^2}{1/100} = (1000 \text{ GeV})^2$$

$$\Lambda \sim 10^3 \text{ GeV} = 1 \text{ TeV}.$$

Naturalness of the electroweak theory breaks down at this scale. If there were no new mass scales, or equivalently no new physics beyond 1 TeV, there was no problem. But that is not so. In general there is no reason to believe that there is no new heavy particle, or new interaction with characteristic scales $> 0(1)$ TeV. In particular, we already know, there is a physical scale, 10^{19} GeV associated with quantum gravity. Thus, natural scale of the electroweak theory, as it stands today, is not 100 GeV but 10^{19} GeV! It is an immediate and serious problem. However, what this hints at is only that there has to be some new physics at and beyond 10^3 GeV so that the standard model with its characteristic scale of 100 GeV becomes natural.

One framework for addressing this problem is to think of the scalar Higgs, not as an elementary particle but, as a fermion-antifermion composite, much in the same way as a pion is made of a quark and an antiquark. This is what is called the technicolour option⁸⁻¹¹. Technicolour is the name given to the new postulated QCD-type force, again a gauge interaction, that would keep these new fermions, techniquarks together in the Higgs particle.

Thus, if we were to probe the Higgs particle with energies greater than 10^3 GeV, we would see it not as an elementary scalar particle, but as a techniquark and a techni-antiquark. Since theories with only fermions and gauge fields are natural like the electrodynamics, now there is no naturalness problem. The condensation of techniquarks results in the masses for weak gauge bosons W^\pm and Z^0 . This still does not solve the problem of masses for the quark and lepton. For that a new gauge interaction, the extended technicolour is introduced. The extended technicolour gauge bosons with masses of the order of 10–100 TeV connect the ordinary quarks and leptons with the techniquarks. This provides a mechanism for the masses of quarks and leptons. However, there are some serious phenomenological difficulties with this scenario. The difficulties include the non-observation of large flavour changing neutral current effects, heavier Higgs particle, absence of large anomalous contributions to the $Zb\bar{b}$ vertex and large contributions to S , T and U parameters^{10–12}.

Another perhaps more attractive framework for addressing the naturalness problem of the electroweak theory is supersymmetric^{13–17}. This option retains the elementarity of the scalar field. In a supersymmetric theory, the naturalness violating effects due to bosonic and fermionic quantum fluctuations cancel against each other. Since this cancellation has to operate at all orders of perturbation, we need a symmetry which relates the bosonic effects to fermionic effects. That is what supersymmetry does: it relates bosonic and fermionic degrees of freedom.

Supersymmetry^{18–20} requires that bosons and fermions come in families. For example, the photon has a superpartner, a neutral fermion, the photino; the electron is accompanied by a scalar partner, the selectron; quarks have scalar partners, squarks; the weak gauge bosons, W^\pm and Z have fermionic partners, the wino and zino, etc. Similarly, if we are studying gravity, spin 2 graviton has a superpartner, a spin 3/2 fermion, so-called gravitino.

Exact supersymmetry would imply that all the properties except the spin of particles in a supermultiplet are the same. Thus, the masses and couplings of superpartners would be exactly equal. This, however, is not borne out in Nature, otherwise we would have already seen, say, the selectron, a scalar electron with the same mass and charge as the electron. Hence supersymmetry must be broken so that the superpartners are heavy enough to have been beyond any detection so far. This breakdown should be such that the basic reason for introducing supersymmetry, namely the naturalness problem, does not get out of hand again. In fact the cancellation between the bosonic and fermionic quantum fluctuations need not be exact, it should be only up to the naturalness breakdown scale of the standard model:

$$m_{\text{particle}}^2 - m_{\text{particle}}^2 \leq (10^3 \text{ GeV})^2.$$

Thus what is required is only a TeV scale supersymmetry. Then, the masses of the particles would be less than one TeV. Present and next generation colliders may, therefore, be able to discover supersymmetry.

The minimal supersymmetric extension of the standard model consists of adding supersymmetric partners to the field content of the standard model. For anomaly-free extension, it also contains two Higgs doublets instead of one. All renormalizable supersymmetric interactions consistent with various global conservation properties are included. In addition, the most general soft-supersymmetry breaking terms (with mass parameters associated with them of the order 1 TeV or below to meet the naturalness constraint) are added.

Besides, the compelling naturalness arguments developed in early eighties^{13–17}, there are other reasons that have emerged since then in support of supersymmetry^{12,21}. Some of these are:

(i) Phenomenologically, a major development hinting at supersymmetry is the beautiful quantitative agreement of precision measurements of the low-energy electroweak coupling constants with the predictions of supersymmetric unified theories. Non-supersymmetric alternative extensions of the standard model have difficulties in this regard.

(ii) Unification of electroweak and strong nuclear forces is possible only in supersymmetric framework; the three coupling constants tend to meet in a point as we go up in the energy scale only in the minimally supersymmetric standard model. We shall return to this point again later.

(iii) There are cosmological implications of supersymmetry. For example, in the minimal supersymmetric standard model, there is a light supersymmetric particle (LSP) which has qualitatively neutrino-like properties. This would be a satisfactory candidate for cold dark matter.

These positive hints are surely tantalizing. Are these mere coincidences? Are there somewhat more definite ways in which we would know if TeV scale supersymmetry is indeed relevant or otherwise? Two possible ways are:

(a) As mentioned above, in supersymmetric extensions of the standard model, there are more than one Higgs doublet. For the lightest Higgs particle, there are rigorous upper bounds, independent of the detailed assumptions, in these models. This is not so in the non-supersymmetric standard model. The numerical value for this bound in minimal supersymmetric standard model is about 150 GeV. For more complicated models (with broken R -parity), this value may go up to 175 GeV. Thus, if no Higgs particle is seen experimentally up to about this upper limit, it is a signal that TeV supersymmetry may not be operative in Nature.

(b) Minimal supersymmetric extension generically tends to reduce the value of quantum corrected parameters, such as couplings, towards the classical (tree) values as compared to those in the non-supersymmetric theory. This is due to the approximate Bose–Fermi cancellations in the loop effects. Thus, in the more detailed experimental precision tests, if the values of the low energy electroweak couplings tend to deviate systematically from the non-supersymmetric theoretical values towards the tree values, this would be a strong hint of minimal supersymmetry.

In the end, only experimental discovery of the superpartners will be the complete vindication of the supersymmetric ideas.

Unification dogma

The oldest example of unification is Maxwell's theory of electromagnetism which provides a combined description of electricity and magnetism. $SU_L(2) \times U_Y(1)$ electroweak theory is a mixed theory of electromagnetic and weak forces. There have been various attempts at developing unified theories of electroweak forces and strong nuclear forces^{22,23}. The most popular one is where three groups of the standard model, $SU_C(3) \times SU_L(2) \times U_Y(1)$ are embedded in a larger group, $SU(5)$. All these attempts at grand unifications imply new mass scales much above 1 TeV. As we have argued earlier, mass scales above 1 TeV make the standard model scale perturbatively unstable. It is, therefore, clear that the unification of these three basic forces can be achieved consistently only in a supersymmetric framework. Further, as alluded to earlier, there is a remarkable property associated with the running coupling constants of the minimal supersymmetric extension of the standard model. The three coupling constants $\alpha_1, \alpha_2, \alpha_3$ associated with the gauge groups $U(1) \times SU(2) \times SU(3)$ respectively evolve with the energy in such a way as to meet at a point only in the minimal supersymmetric standard model and not in the non-supersymmetric standard model²⁴. The meeting point occurs at $\mu = 2 \times 10^{16}$ GeV. It is a clear hint of unification at about 10^{16} GeV in the supersymmetric case. Above this scale, all the three interactions are the same and are governed by a single coupling constant corresponding to gauge group $SU(5)$.

In this $SU(5)$ unification, the quarks and leptons are members of one family. The differences which are seen between quarks and leptons are to be viewed as the low-energy phenomena obtained by spontaneous breakdown of the larger $SU(5)$ symmetry to the smaller $U(1) \times SU(2) \times SU(3)$ symmetry at the unification scale of about 10^{16} GeV. In this scenario, there are gauge interactions between the quarks and leptons me-

diated by new gauge fields, the lepto-quark gauge bosons of $SU(5)$. These new gauge bosons are very heavy, with masses given by the unification scale with the result that the new interactions are very weak. One important implication of these new interactions is that proton in these models is not completely stable. Its lifetime is large but finite, $\tau_{\text{susy}} \sim 10^{35} - 10^{39}$ years in the supersymmetric $SU(5)$ theory, as compared to $\tau_{\text{non-susy}} \sim 10^{27} - 10^{31}$ years in the nonsupersymmetric $SU(5)$ model. Experimental limits for proton lifetime are $\tau_{\text{expt}} > 10^{32}$ years, clearly in contradiction with non-supersymmetric model, but consistent with the supersymmetric version. This is satisfying from the supersymmetric point of view. It need not have been so. This surely is not evidence for supersymmetry, but, if the proton lifetime had not worked out to be long enough, it would have been a clear evidence against minimal supersymmetric $SU(5)$ unification.

The unification scale for the three interactions, the electromagnetic, weak and strong, $\mu \sim 10^{16}$ GeV is only three orders of magnitude away from the scale of quantum gravity, $\mu_{\text{Planck}} \sim 10^{19}$ GeV, at which the quantum features of gravity become relevant. It may as well be that we should be thinking of unification of all the forces, including gravity, at the same time. Here again supersymmetry offers an advantage. So far we had been discussing supersymmetry which holds in the same way everywhere in space-time, the global supersymmetry. We could also think of supersymmetry which holds independently at every point of space-time, the so-called local supersymmetry. Gravity is automatically included in such theories. These theories are called supergravity theories. Here spin 2 transverse graviton has a partner, spin 3/2 gravitino. Supersymmetric matter can also be added to these theories. Recall we want a good quantum theory of gravity to be finite. Unfortunately, supergravity theories still are not satisfactory from this point of view. Thus we can think of supergravity theories only as effective low energy theories. As we go up in the energy scale up to the Planck scale, the supergravity theories would have to be replaced by some other fundamental theory which would have to be finite. There do exist candidates for such a theory. These are the superstring theories.

In a string theory²⁵, the elementary entities are not point particles, but tiny linearly extended objects of the size of Planck length, 10^{-33} cm. Strings can be of two types: open strings and closed strings. Ordinary matter like electrons, quarks, photon, gluons, etc. and their superpartners are described by an open superstring. Graviton and its superpartner gravitino are identified with a closed superstring. This identification respects the basic interaction properties of these particles, namely all types of matter experience the gravitational forces, but graviton does not experience the non-gravitational forces. This property is reflected in the fact

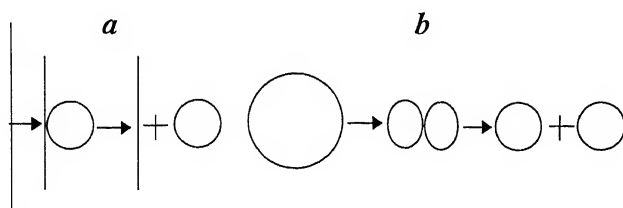


Figure 1a, b. An open string emits an open string and a closed string but a closed string emits only closed strings.

that an open string can emit a closed string as in Figure 1a, but a closed string can emit only a closed string, not an open string, as in Figure 1b.

This picture is indeed encouraging. In fact it does turn out that a closed string does have a spin 2 massless excitation to be identified as the transverse graviton, and open string does have massless spin 1 excitations which are identified with transverse vector gauge bosons.

Some of the features of the string theories are²⁵:

(i) This is a framework where all the fundamental forces, including gravity, are unified. It also unifies the matter (quarks and leptons) with mediators of the basic forces (gauge fields), as all these are supposed to be excitations of the same string. That is why sometimes, the string theory is pompously advertised as the *Theory of Everything*.

(ii) Superstring theory provides a *consistent* and *finite* theory of quantum gravity. It is for the first time that principles of general theory of relativity and quantum physics have been consistently married.

(iii) Even if, in the end, it does not turn out to be a completely satisfactory description of Nature, it does provide a theoretically rich testing ground for important conceptual issues of quantum gravity, particularly those related to the fate of black holes.

(iv) Supersymmetry is essential for this theory. Without supersymmetry, string theory is not consistent; it has a tachyon in its spectrum and also it does not provide a finite theory of gravity.

The superstring theory is also beset with difficulties. One problem is that there are several versions of the theory with no clear indication at present as to which of them is preferred by Nature. There is no understanding of the mechanism of supersymmetry breaking both in the supersymmetric field theories as well as in the superstring theories. These issues are expected to be related to the non-perturbative behaviour of these theories. There have been some recent developments which have allowed significant progress in this direction. A lot of circumstantial evidence is emerging for an electric-magnetic type of duality in these theories which relates any string theory at strong coupling to another string theory at weak coupling²⁶⁻²⁸. This may point towards the fact that there is only one fundamental theory of which the various superstring theories are only the asymptotic limits.

Though superstring theory provides a rich and conceptually deep framework, a major difficulty with it is that there is at present no easy way to test whether it is a correct description of Nature. Experimentally-useful effects of strings would occur typically at 10^{19} GeV. There is a huge gap between the present and near future experimentally reachable energy of 10^3 GeV and the Planck scale of 10^{19} GeV where the stringy features manifest. How can this gap be bridged or substantially narrowed?

Summary

We have explored the four dogmas of theoretical high energy physics, namely gauge principle, renormalizability (or finiteness for gravity), naturalness and unification. The requirement that the standard model be natural beyond 1 TeV leads to the extraordinary conclusion that Nature should be supersymmetric at that scale. This is experimentally interesting, because it implies possible supersymmetric particles just around the corner from the energies being explored at present. Discovery of supersymmetry is one of the foremost tasks of the machines of the next two decades. Supersymmetry, if discovered, will open up a whole variety of new particles and phenomena to be studied and analysed. This will influence profoundly the kind of physics that will be done in the 21st century. Supersymmetry may even have implications for cosmology.

Requiring a finite quantum theory of gravity and grand unification of all the four fundamental forces leads us to the speculative, but theoretically rich and conceptually challenging framework of superstrings. Supergravity theory would be only a low energy (at energies $\ll 10^{19}$ GeV) effective theory of such a superstring theory. While TeV supersymmetry will perhaps be discovered experimentally in the next decade or so, let us hope that some evidence, even if cloudy, for stringy ideas may also emerge early in the 21st century.

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New ideas on acceleration to Planckian energies

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A plasma can sustain electric fields that are many thousands of times stronger than those of the most powerful present-day conventional particle accelerators. Plasma-based accelerators thus offer exciting new possibilities and point towards superhigh energies in the future – a promising first step towards Planckian energies.

THE primary motivation for building particle accelerators of ever-increasing energy has come from high energy physics. Starting from the thirties when cyclotron accelerators generating energies of a million electron volts (MeV) provided the necessary tools to study nuclear reactions in the laboratory, the modern day synchrotrons and linear accelerators of up to trillion electron volts (TeV) are helping us probe the fundamental forces of nature and understand the conditions of the early universe. They provide the only controlled and direct means of testing theoretical models, such as the standard model, and explore questions and problems beyond the realm of these models. Unfortunately, the conventional accelerator technology is approaching practical limits and cannot take us to the energy range of interest to high energy physics in the near and long-term future. What are these energies? In the near term, the interest lies in the 10 TeV–100 TeV range where deviations from the standard model can be tested. And in the long term if quantum gravity, the ultimate frontier of high energy physics has to be explored, then one must attain Planckian energies which are of the order of

10^{19} GeV. Conventional accelerators certainly cannot take us there. Infact, the operating principle on which the present-day accelerators are based is about half a century old and one has more or less reached the limits of technology here. Basically these accelerators use strong magnetic fields to guide particles which are propelled by strong electric fields created in vacuum by RF sources. The guide field cannot be raised substantially since they will exceed the structural forces of the magnetic materials used and the electric field strengths are likewise limited by material breakdown limits. The maximum electric field one can obtain is about 1 MV/cm, i.e. 100 MV/m. Thus, to accelerate particles to 10 TeV, one needs to construct an accelerator that is about 100 km in length. The enormous capital costs and the engineering complexities involved in building such devices considerably diminish their future viability. The cancellation of the Superconducting Supercollider (SSC) is a telling example of the kind of fate that can befall such devices. It also underscores the need to come up with new ideas and look for alternative schemes.

Fortunately, plasma particle acceleration, a new technology that has made rapid advances in the past few years, offers a promising alternative. A plasma is a state of matter which is at a temperature where all the atoms are completely ionized. Such a state has overall charge neutrality but local imbalances in charges can give rise to large longitudinal electric fields. These fields, which cause the plasma electrons to oscillate back and forth around the massive ions – the so-called plasma oscillations – can be effectively used for particle acceleration.

Unlike in a conventional accelerator, these are not dc fields – in fact a plasma strongly opposes the existence of dc fields – but one is talking here of oscillating fields due to travelling waves. Thus, to accelerate particles one has to have them moving nearly at the speed of the wave so that in the moving frame the fields appear to be almost dc. Since a plasma is already an ionized medium, it is not subject to any further electron dissociation (electrical breakdown) and hence the accelerating electric fields can, in principle, be thousands of times stronger than what conventional accelerators can provide. Typically, the maximum electric field is proportional to the amplitude of the charge imbalance (fluctuation) and to the square root of the plasma density. Plasma densities in the range of 10^{16} cm^{-3} to 10^{21} cm^{-3} are not difficult to produce in the laboratory and the maximum electric fields that they can sustain can therefore be of the order of 100 MV/cm to 30 BV/cm. These are about a factor of 10^3 superior to conventional RF systems. With such fields a plasma accelerator, of only a few 100 metres length, can produce the acceleration energies of the 87 km SSC!

How does one create these fields in the plasma to accelerate particles? I will discuss here the basic principles of two promising methods – the Beat Wave Accelerator and the Wake Field Accelerator. I will summarize their theoretical and experimental achievements to date and briefly discuss their prospects as practical accelerator devices of the future.

Plasma beat wave accelerator

The basic idea of plasma-based accelerators relies on the generation of electrostatic plasma waves travelling close to the velocity of light. Such plasma waves can then readily accelerate electrons to the required energies. In the plasma beat wave scheme, such waves are excited by mixing two collinearly propagating laser beams with frequencies ω_1 , ω_2 and wave vectors \mathbf{k}_1 , \mathbf{k}_2 such that their frequency difference matches the plasma frequency, i.e. $\omega_1 - \omega_2 = \Delta\omega \sim \omega_p$. The electrons respond resonantly to the beat frequency and give rise to large plasma oscillations. The wavenumber of the plasma wave is given by $k_p = \Delta k = \mathbf{k}_1 - \mathbf{k}_2$ and its phase velocity $v_{ph} = \omega_p / k_p$ is then equal to the group velocity of light in plasma $v_g \approx \Delta\omega / \Delta k \approx c(1 - \omega_p^2 / \omega_1^2)^{1/2}$ if $\omega_1, \omega_2 \gg \omega_p$. If a charged particle is injected into this wave at approximately the same velocity as the wave then it will stay in phase with the field, absorb energy from it and steadily accelerate. The process is analogous to a surfer gaining energy from an ocean wave as he rides the wave and slides down its slope. The idea of the beat wave scheme was first put forward by T. Tajima and J. M. Dawson¹ in a classic paper in 1979.

The strength of the longitudinal electric field E can be estimated approximately from Gauss's law, $\nabla \cdot E \approx ik_p E = n_1$ where n_1 is the oscillating density perturbation. This gives the result

$$E = |\phi k_p| \approx 0.96 \epsilon \sqrt{n_0} \text{ V/cm},$$

where ϕ is the wave potential, $\epsilon = n_1/n_0$ is the plasma wave amplitude and n_0 is the plasma density expressed in cm^{-3} . An electron falling through this potential will gain an energy ΔW given by

$$\Delta W \approx 2\epsilon \gamma_{ph}^2 mc^2,$$

where γ_{ph} is the relativistic Lorentz factor $\gamma_{ph} = (1 - v_{ph}^2/c^2)^{-1/2} \approx \omega_1/\omega_p$. Substituting for γ_{ph} we see that

$$\Delta W \approx 2\epsilon mc^2 \omega_1^2 / \omega_p^2.$$

Thus for a chosen frequency ω_1 the accelerating gradient E is maximized for large ω_p but the corresponding maximum energy gain ΔW is reduced due to the inverse dependence on ω_p . As the electron gains energy from the wave, it slips forward in phase and this dephasing limits the maximum energy gain. The length over which this dephasing occurs is given by

$$L \approx \frac{\Delta W}{eE} = 2\gamma_{ph}^3 / k_0.$$

The energy limit due to dephasing can be overcome somewhat if the electrons move at an angle to the direction of the wave propagation – again much like a surfer riding a wave at an angle to get a longer ride and move faster. The electrons can be made to move at an angle by applying a magnetic field perpendicular to the plasma wave's direction. Other factors that can limit the interaction length are diffraction of the laser beams, pump depletion of the laser beams and turbulence effects. At high laser intensities the plasma dielectric properties favour a self-focusing of the laser light, thus compensating for the diffraction effects. Likewise, the radial electric fields of the plasma waves help keep the accelerated electrons in focus. The accelerated electrons also give rise to a strong current which produces strong confining magnetic fields and aid the unimpeded progress of the electrons along the axis. Laser-induced turbulence, which impedes the formation of plasma waves, can be minimized by shortening the laser pulse length. The idea is to operate on time scales over which the ions remain virtually stationary so that ion sound waves cannot be excited. With the advent of nanosecond (and now picosecond) laser pulses, it is possible to avoid plasma turbulence over extended lengths. Many of these phenomena have been extensively investigated and tested in computer simulations.

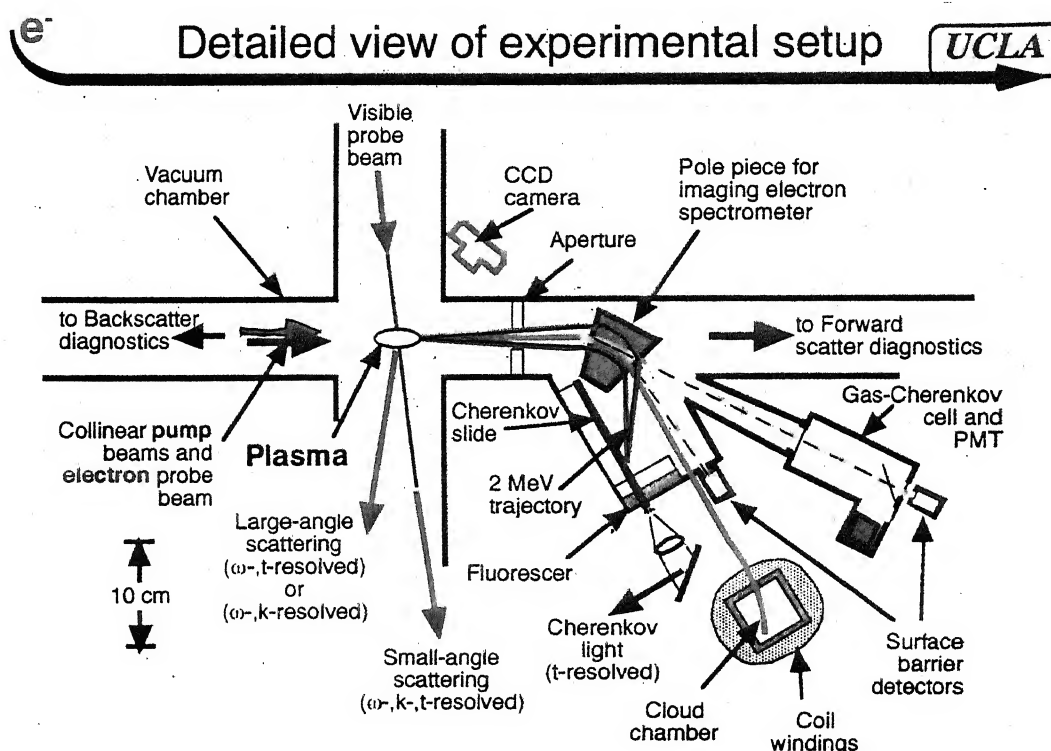


Figure 1. Schematic of the UCLA Plasma Beat Wave Accelerator experiment (courtesy C. J. Joshi).

There have also been a large number of experiments on the beat wave scheme starting almost from the time of its first proposal in 1979. One of the leading contributors is the Indian physicist C. J. Joshi, who began his early experiments in Canada and then achieved some spectacular results in a series of experiments at UCLA². The initial experiments established the generation of large-amplitude plasma waves. Subsequently acceleration of externally injected electrons to progressively higher and higher energies has also been observed. In the UCLA setup, typically, a two-frequency beam from a 200-GW CO₂ laser (with wavelengths of 10.5 and 10.2 μm) is focused into the plasma as shown schematically in Figure 1. A 2-MeV electron beam generated in a linac is passed through this plasma region. The temporal and spatial properties of the plasma oscillations generated by the beating laser waves are studied by optical scattering techniques. The electric fields are directly probed by measuring the energy gain or loss of the electrons. A maximum energy gain of 28 MeV corresponding to an acceleration rate of 2.8 GeV m⁻¹ has been observed³. The density perturbation of the plasma wave is about 23% and the ambient density is around $9 \times 10^{15} \text{ cm}^{-3}$.

Plasma wake field accelerator

One of the major technical complexities of the Beat wave Accelerator Scheme is the resonant matching

condition, which requires that the frequency difference of the two laser beams be equal to the plasma frequency. This puts severe constraints on the required uniformity of the plasma. Nonuniformities because of ambient electron density gradients or because of random density fluctuations cannot be tolerated. An alternative scheme, called the Laser Wake Field Scheme is a more rugged and simple method in which plasma waves are created by a sudden impulse with a short and intense laser pulse. The laser pulse rise time is of the order of a plasma period. Just as a speeding boat pushes the water aside at the prow and leaves a wake behind it, the laser pulse pushes aside the plasma electrons which rush back at the exit of the pulse and give rise to plasma oscillations. This plasma wake travels at the group velocity of the laser pulse. Electrons placed in the wake field can then be accelerated in the same fashion as discussed in the previous section. Wake fields can also be generated in a plasma by using a short bunch of energetic electrons instead of a laser pulse – it is then called the electron beam wakefield accelerator. For optimum gain it is necessary to tailor the driving beam profile so that it has a slow rise (over many plasma periods) followed by a rapid falloff.

The wakefield concept has also been extensively studied in computer simulations and detailed theoretical analyses. Two of the key questions in this scheme relate to the nature of the nonlinearly coupled electromagnetic and plasma wave excitations in a cold plasma with rela-

tivistically intense laser fields and the typical group velocity of such structures in the plasma. In this context, some exact one-dimensional nonlinear solutions for modulated light pulses coupled to plasma waves have been recently analysed⁴. The solutions are in the form of soliton pulses which may be viewed as a light wave trapped in a plasma wave that it generates itself. The front of the pulse generates the plasma wave, which is then reabsorbed by the tail of the pulse. Such pulses are not only of interest for particle acceleration but can be used for photon acceleration as well. Numerical results also give a fairly accurate estimate of the group velocity of these pulses and elucidate the nonlinear relationship between the group velocity, amplitude and frequency of the wave. Some of the other limitations discussed for the beat wave accelerator e.g. diffraction effects, pump depletion, etc. also apply to the wake field accelerator. Ideas such as relativistic guiding and plasma density channels have been proposed for overcoming diffraction effects. A novel idea that has been recently proposed⁵ envisages the use of an active medium that can not only continuously replenish the loss of the laser energy to the wakefield (thereby eliminating pump depletion) but also accelerate the group velocity of the pulse to a desired value (thereby maintaining phase resonance with the trapped electrons).

Early experiments on the wake field accelerator (using electron beams as drivers) were done by David B. Cline *et al.*⁶. Direct observations of the laser wakefield accelerator have recently been reported by Roger Falcone and his colleagues⁷. Experiments at the Institute of Laser Engineering⁸ at Osaka report generating laser wakefields with gradients of 30 GV/m over a distance of 0.6 mm.

Future prospects

Plasma-based accelerators are an active area of research today and a great deal of theoretical and experimental work is in progress. Several variants of the above two basic schemes, such as the inverse free electron laser, the inverse Cerenkov accelerator, laser driven grating Linac, etc., also exist and are receiving increasing attention. The beat wave scheme is probably the one that has been the most actively pursued. Major experiments are being carried out at UCLA in the USA, the Imperial College, London and the Rutherford Appleton Laboratory, Didcot in the UK and at the Ecole Polytechnique, Palaiseau in France. In a recent collaborative experiment between these centres, gradients of 100 GV/m were observed (maximum measured energy gain of 44 MeV in 350 μm). This is by far the highest collective-wave field ever produced in the laboratory.

In view of all this recent experimental progress, the prospects of accelerating a significant number of electrons to one GeV energy in the near future appear very

Table 1.

Laser wavelengths	1.05 μm and 1.06 μm
Plasma density	10^{17} cm^{-3}
Plasma source	Multiphoton ionization
Laser pulse length	4 ps
Laser power	14 TW
Laser spot size (2σ)	200 μm
Rayleigh length (Z_0)	3.1 cm
Plasma homogeneity	$\pm 7\%$
Peak plasma wave amplitude	0.5
Peak gradient	160 MV/cm
Final energy	1 GeV

promising. The technologies associated with the laser, plasma and the electron beam injector are available and the key issues related to plasma production, plasma wave excitation, control of instabilities and optimization of the acceleration process appear to be sufficiently well understood. On the basis of this progress, there has recently been a proposal⁹ to construct a 1 GeV plasma beat wave accelerator by the UCLA group. Table 1 lists the principal parameters of this proposed accelerator. The main goal of this experimental accelerator would be to demonstrate the acceleration of a substantial number of electrons (of the order of 10^8) to about 1 GeV energy with a reasonable energy spread without the need to employ laser beam guiding.

If this experiment is funded and if the pace of present progress continues, it is not unreasonable to expect the construction of 500 GeV machines based on plasma concepts within the next decade. What about Planckian energies? The plasma accelerators that we have just discussed and which rely on electrodynamics certainly cannot get us there. The maximum laboratory-produced plasma densities we can expect are of the order of 10^{27} cm^{-3} which could yield electric fields of the order of 10^6 GV/m . So to achieve an energy gain in the Planckian regime (i.e. 10^{19} GV) we would need an accelerator of 10^{13} m length! We, therefore, need to make a few more quantum leaps and dream up some more crazy ideas. What about tapping Quark Gluon Plasma (QGP) fields? Typically, if one considers the energy density in a hadron to be of the order of 2 GeV-fermi^{-3} , then the colour fields which can be of the same order of energy density can be estimated to be about $\sim 3 \times 10^{14} \text{ GV/m}$. Of course, such fields would only accelerate confined particles (quarks) but if one argues that the colour fields would also be strongly coupled to electrodynamic fields, then one is talking of very large electric fields indeed which could be used for electron acceleration. The energy gain would be severely limited though by length constraints (extent of the QGP). One can well speculate, therefore, about an entirely new technology – a QCD-based technology¹⁰. Such a development is not unconceivable and may happen sooner than we imagine. Meanwhile, plasma accelerators have

certainly shown us a path to overcome the limitations of the present accelerator technology and move towards multi-TeV energies. This should serve as an encouragement to dream of higher goals.

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High Energy Physics in the 21st century – A summary

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NOT too long ago there was a startling suggestion by Stéphen Hawking asking whether the end of Theoretical Physics was in sight. This was soon after the euphoria that accompanied the emergence of the string theory as a possible Theory of Everything and a feeling that there was now a satisfactory explanation for at least all of basic issues. After about ten years, we now perceive that unless new ideas for particle acceleration emerge, experimental high energy physics may be at an end. And that indeed can have a deleterious effect on theoretical High Energy Physics as well.

Particle Physics is, indeed, on important cross-roads at the moment. As described by D. P. Roy, we now have a standard model, with almost all experimental data in the energy range up to about TeV (10^{12} eV) accounted for, by means of about 20 parameters in a quantum field theory with adequate local symmetry. There are many hints as to what lies beyond the standard model and it is only to be expected that the situation will become clear once the experiments, currently being pursued, provide the necessary data.

As a check list, it is worth drawing up a collection of an immediate set of problems for the early 21st century as was done by Gross, Witten and Kane as a part of their assessment of outstanding questions:

(i) What determines the gauge symmetry at ordinary (?) energies (1 TeV) to be $SU(3)_c \otimes SU(2) \otimes U(1)$; $SU(3)$ signifying quantum chromodynamics and $SU(2) \times U(1)$, the unified electroweak theory of Salam and Weinberg?

(ii) How will gravity enter this picture? Through superstrings?

(iii) What is the nature of unification of the familiar forces? Is there a grand unified gauge symmetry group relevant at higher energies? Is such a unification a forerunner to incorporating gravity?

(iv) What constrains the quantum numbers of quarks and leptons? Why are the left-handed and right-handed fermions different? Is there a fundamental reason for us to have 'chiral' fermions?

(v) Why do we have different families or generations of fermions? How many? (The old version of the same question was asked by Rabi: Who ordered muon?)

(vi) What is the physics of Yukawa coupling? (What determines the masses and mixing angles of the quarks and leptons?)

(vii) Most abundant constituents of all matter are (u , d) quarks and electrons. Why are they so light in comparison with W^\pm , Z , top quark, Higgs (?) which appear to be in the 100 GeV range, presumably the natural scale of the theory.

(viii) Why is the vacuum energy (cosmological constant Λ) vanishing? How can we ensure this when the supersymmetry (boson \leftrightarrow fermion symmetry) is broken, as it indeed must.

We can be sure that as we provide answers to these issues, new queries will arise.

We observe that the main theoretical tools, that we now use, to answer the many puzzles, appear to be tak-

ing a somewhat universal shape; seem to be endowed with abilities to answer many varieties of phenomena in diverse fields. And this is a welcome trend. There has been considerable sharpening of the ideas in relativistic quantum field theory and symmetry principles. Kaul has described how supersymmetry is helpful in explaining certain notions of 'naturalness' and how different energy scales coexist in our theoretical framework. Supersymmetry, appears to have special virtues. It helps tame infinities in theories with scalar fields, which in turn are necessary for spontaneous symmetry breaking by Higgs phenomenon since fermion and boson loop divergences are negative of each other and protect the hierarchy of energy scales. Further, it provides a natural explanation for the vanishing of the cosmological constant (since there is a neat cancellation of all zero point energy in a supersymmetric quantum world). Perhaps direct evidence for supersymmetry is just around the corner.

One of the exciting developments in the closing stages of the twentieth century is the emergence of the string theory. This has turned out to be an incredibly rich framework and the string experts feel that it will have applications not only in understanding basic aspects of High Energy Physics, but will be of value in many other disciplines as well. Among its properties are many new hidden symmetries. It comes endowed with many kinds of dualities – for instance, indicating a relationship between one string theory with a coupling parameter g with another with a coupling parameter $1/g$. This opens up a possibility of understanding the strong coupling regime of one theory by looking at the weak coupling (and hence well understood) aspects of another related string theory. What is more, string theories have greater control over singularities by virtue of the presence of a natural length scale in it. Further, superstring theory, which incorporates supersymmetry as well, has ingredients of a renormalizable field theory of all interactions that encompasses all known forces of interaction and species of matter. The versatility of the theory is such that it has resulted in hitherto unknown proofs in the geometry of manifolds and this has made an impressive impact in some areas of pure mathematics.

On the experimental side, Particle Physics has always depended on large projects involving many institutions and a very large number of physicists. We seem to need gigantic accelerators (and hope to reach 200 GeV in e^+e^- collisions by the turn of the century and make a jump from 2 TeV for $p\bar{p}$ at Fermilab tevatron to 14 TeV at the CERN Large Hadron Collider in the first decade of the next century) and complex detector systems that go with them. The accelerator-based HEP is supplemented by the 'underground' labs to do precision measurements making use of solar and atmospheric neutrinos and other exotics and further look for signals from astroparticle physics-related observations. In the 21st century, the need will be to push to higher energies as well as to

higher precision. Both require innovative efforts as pointed out by Cowsik and Sen.

There is much concern that the projects in High Energy Physics are getting bigger and more expensive. SSC (Superconducting Super Collider) at Texas had to be abandoned as unaffordable. There is a perception of growing fatigue in the support of science for science-sake. It is suggested that there are more deserving claimants for the public support and there is a perennial debate between 'big' science and other sciences. It is unfortunately forgotten that the quest for new acceleration techniques and new principles of particle detection, identification and computing, etc, will have a direct impact not only in High Energy Physics, but in a whole variety of disciplines. It is inescapable that there will be direct consequences for: Condensed Matter Physics, basic as well as applied areas; advances in information technologies/computing/data handling (WWW – the world wide web of distributed information network had originated from CERN); and many aspects of Material Science. Indeed, Experimental Particle Physics may be the most efficient way to develop high-tech applied sciences.

It is with this background that we should review the programme of Experimental High Energy Physics in India. In the past, our efforts had banked on (i) cosmic ray experiments (ii) analysis of emulsion stacks and bubble chamber data from High Energy Physics experiments elsewhere (iii) KGF underground laboratory for proton decay and ν interactions and (iv) experiments at CERN and Fermilab. At present, we play important roles in both CERN LEP experiments (L3 collaboration) and Fermilab efforts (DØ collaboration). In the future there are plans for active participation in the Large Hadron Collider project at CERN ($p\bar{p}$ collider at centre of mass energy of about 14 TeV in the LEP tunnel) both at the stage of construction of the accelerator and later on in doing physics experiments with it.

We should, I believe, think in terms of supplementing these efforts by activities based in India. It will be useful to initiate thinking about various options we may pursue. For instance, let me start with a short list for active consideration:

- (i) A new innovative 'underground' laboratory, with international funding and participation.
- (ii) A new task force (think tank?) as suggested by Rajasekaran for new principles of particle acceleration (Planckian?).
- (iii) A time-bound programme for building a high energy accelerator in India, say in the range 10 GeV–20 GeV, targeting a specific niche. The aim should be for a unique device, say, for example, to study high precision polarization (beam/target) phenomena. We may thus look forward to having a component of High Energy Physics activity based in India in the not-too-

distant future, so that our national efforts complement our commitment to the collaborations elsewhere.

To conclude, what, may we expect, the shape of 21st century High Energy Physics to be? It is worth recalling that in the early sixties, particle physicists *expected* (i) to reach the region of asymptotic flat cross-sections, signifying the diffraction scattering of strongly interacting particles as being due to dominant Pomeron exchange at 60 GeV (at that time, next high energy (ISR: intersecting storage ring) machine), and the culmination of Regge Theory; and (ii) made bold predictions that the intermediate vector bosons that mediate weak interaction

could be as heavy as 2 GeV! However we now find that (i) W^\pm , Z have masses 40 to 46 times heavier! and (ii) there is no hint of asymptopia, but we have something much better. A gauge theory of strong interaction described by QCD!! Regarding the future, therefore, it would be hazardous to make any guess; maybe the space-time will be granular and new paradigms will begin to take shape. Perhaps any guess that we now make may not be wild enough.

I would like to, nevertheless, believe that 'particle physicists are grappling with wonderful questions and marvelous and mysterious ideas'. No marks for guessing who said this.

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A new technique for visualization of shock shapes in hypersonic shock tunnel

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Based on the principle of dependence of the intensity and the spectrum of the spontaneous light emitted in an electrical discharge on the density and the temperature of the gas along the discharge path, a technique for flow visualization around the bodies flying at hypersonic Mach number is developed. The shock shapes over a flat plate with sharp leading edge at different angles of attack are visualized using this technique in IISc hypersonic shock tunnel HST1 at flow Mach number of 5.75. The visualized shock wave angles match very well with the theoretical values. The details of this new technique along with a few sample results are presented here.

THE flowfields around hypersonic vehicles are very complex and measurement of these flowfields is important for their understanding. The flow visualization is

conventionally achieved by employing optical systems, such as the schlieren method, interferometry, shadowgraphs and holography. Except holography, none of the other systems is useful for three-dimensional shock wave visualization because the visualization in these systems is achieved by passing the light along the optical axes perpendicular to the direction of the gradient of the gas density. On the other hand, the holographic technique is very sensitive to mechanical vibrations and hence is very difficult to use for the flow visualization in the wind tunnel tests.

A simpler technique based on the electrical discharge for the visualization of three-dimensional shock shapes around hypersonic vehicles has been reported recently¹. This technique is based on the principle that the intensity of spontaneous radiation emitted by the ion recombination in an electrical discharge depends on the gas density along the discharge path². In this method when an electrical discharge is generated across a shock wave, the light emitted from the shock wave region will be very weak compared to that from the freestream and the shock layer. As a result, the shock shape appears as a dark line in the intensity field. However, the electrical discharge technique has been developed for the flow visualization in a hypersonic wind tunnel where the test time is above 10 milliseconds. In this paper we report a

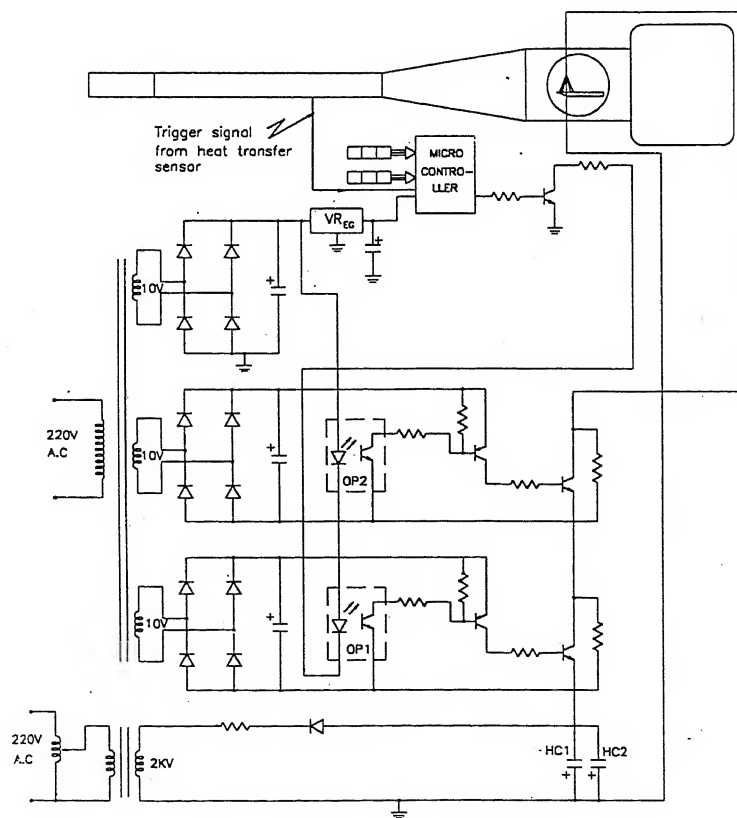


Figure 1. Schematic diagram of a hypersonic shock tunnel with the circuit diagram for the generation of electrical discharge of 2 μ s duration across the pair of electrodes inside the test section.

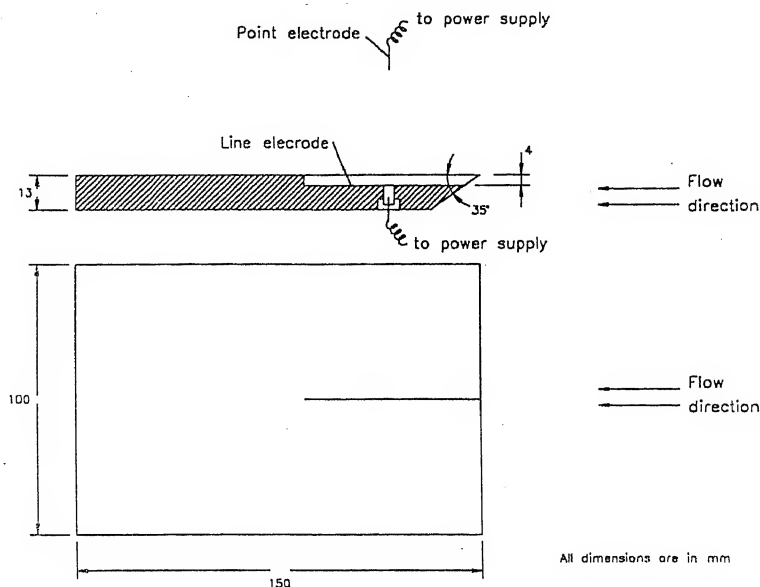


Figure 2. Arrangement of point and line electrodes for the visualization of shock shapes around the flat plate model.

new electrical discharge technique for the visualization of flow in hypersonic shock tunnel where the test time is less than a millisecond. The shock shapes around a flat plate with a sharp leading edge at flow Mach number 5.75 are visualized using this technique in IISc hypersonic shock tunnel HST1. The measured shock angles match very well with the predicted values at various angles of attack.

A schematic diagram of the IISc hypersonic shock tunnel HST1 along with the electrical circuit for the generation of the electrical discharge across the test model is shown in Figure 1. Freestream Mach numbers up to 13 can be generated in this tunnel using a nozzle with appropriate area ratio. A conical nozzle with an exit diameter of 30 cm expands the flow into a 45 cm long test section of 30 cm \times 30 cm area. Two optically clean windows are provided for photographing the discharge phenomenon in the test section.

The electrical discharge is generated across a point electrode and a line electrode. The point electrode of 1 mm dia is suspended in the freestream inside the test section while the line electrode made out of a 0.5 mm thick copper strip is embedded in the test model such that one of its edges is flush with the top surface of the model. The electrodes are kept about 60 mm apart. The test model is made out of bakelite material to ensure insulation of the rest of the tunnel from high voltages and also to avoid multiple reflections from the model surface. The details of the flat plate and the electrode arrangement are shown in Figure 2.

The details of the electrical discharge circuit developed for the purpose of generating discharge between

the electrodes are shown in Figure 1. Since it is critical to ensure the occurrence of the discharge after the flow attains steady state in the test section, a circuit is built for delaying the occurrence of the discharge after receiving the trigger signal with a facility to limit the discharge duration. The trigger signal for the circuit is obtained from a platinum thin film sensor located towards the end of the shock tube mounted flush with the inner surface of the driven section. The sensor generates an electrical pulse corresponding to the rise in temperature due to the arrival of the shock wave. This pulse becomes an input signal to the microcontroller encompassing an 8 bit 8031 microprocessor, a 2764 EPROM and a couple of digital switches. This controller has facility for adjusting a delay and the pulse duration. The delay is carefully adjusted such that the electrical discharge is generated after the nozzle starting process is complete and a steady flow is established in the test section. The delayed signal acts on two optical couplers OP₁ and OP₂, which have an integrated LED and a phototransistor. When activated, the light from the LEDs is sensed by the phototransistors and the photocurrent generated from these diodes will switch on the high voltage transistors BU 205s connected in parallel. This closes the electrical circuit and the high voltage is applied between the pair of electrodes by electrical charge stored in the condensers HC₁ and HC₂ which generates the discharge. After the time lapse set by the pulse duration the microcontroller sends an interrupt signal which will turn off the high power transistors and break the continuity of the electrical circuit terminating the electrical discharge.

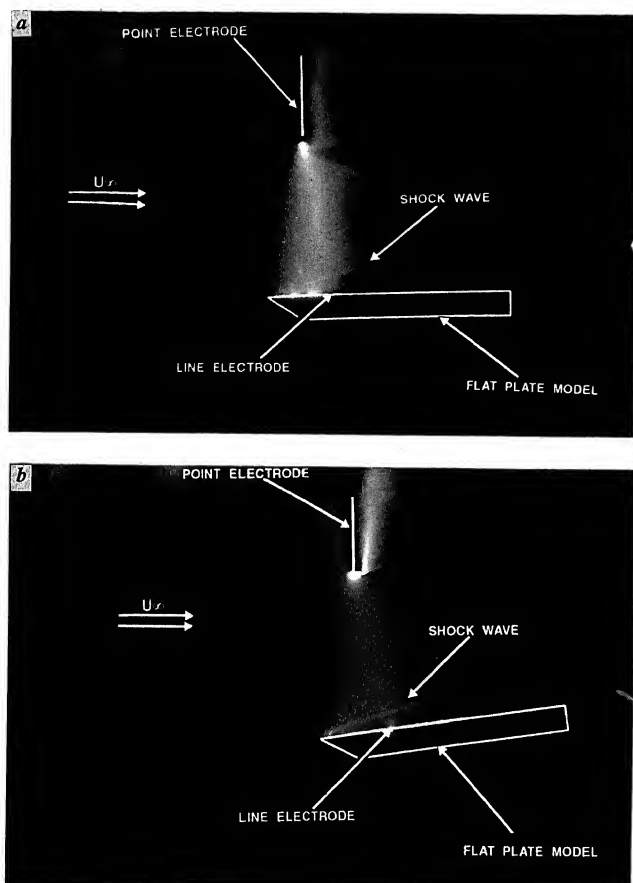


Figure 3a, b. The shock waves over a flat plate with a sharp leading edge inclined at (a) 5° and (b) 6° to the flow direction inside the test section of the HST1 tunnel at Mach number 5.75.

The experiments were conducted with the following test conditions: flow Mach number = 5.75, Reynolds number = $1 \times 10^5 \text{ m}^{-1}$, freestream density = $1 \times 10^{-2} \text{ kg/m}^3$, duration of the steady flow ~800 μs , freestream temperature = 173 K and freestream static pressure = $9.9 \times 10^{-3} \text{ mbar}$. In these experiments the point electrode was used as the anode and the line electrode was used as cathode. The voltage applied across the electrodes was about 1500 V and the electric current was set at 1 A. The discharge in the test section was photographed from outside the side window using a Nikon FM 2.8 camera with B exposure. A film with ASA 1600 speed was used for recording the weak light emitted from the discharge field. Some of the photographs were taken at film speeds of ASA 3200 and ASA 6400 by using the push processing technique in order to record the spectral variation of the light emitted from the temperature layer. However these preliminary experiments did not reveal the temperature layer because the temperature in this layer will be very low for the flat plate with a sharp lead-

ing edge. Currently the efforts are being made to study the temperature layer with a blunt nose cone model.

Typical results of the shock shape visualization are shown in Figure 3a and b for the flat plate at 5 and 6 degrees angle of attack, respectively. Unlike the dark line representing the position of the shock wave in the long-duration tunnels¹, the shock position in these figures is indicated by the bright line. The bright portion at the shock wave occurs in this case because the experimental conditions are suitable to make the electron energy at the shock wave equal to about 20 eV (ref. 3). The measured shock wave angles of 13° and 14° match very well with those theoretically estimated using oblique shock relationship⁴ for both the cases.

In conclusion, we have demonstrated a new technique for the visualization of the shock shapes around the hypersonic vehicles tested in hypersonic shock tunnel. This method is novel as it suits application in the shock tunnel where the typical test times are less than a millisecond. The measured shock angles for a flat plate with sharp leading edge at an angle of attack tested at flow Mach number 5.75 in IISc shock tunnel HST1 match very well with the theoretical results. This technique can be utilized for visualizing the three-dimensional shock shapes by taking the photograph of the electrical discharge either in the downstream or upstream direction of the flow.

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Steric enhancement of resonance: An electron localization perspective

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Steric enhancement of resonance (SER) has earlier been characterized in terms of molecular properties such as dipole moment, bathochromic shift, mesomeric moment, spin–spin coupling constant or by monitoring the kinetics of a reaction. The present work provides a unified picture of SER in terms of the electron localization patterns in a molecule.

THE phenomenon of steric hindrance of resonance (SHR) has been well documented¹ in the organic chem-

istry literature for a long time. However, that of steric enhancement of resonance (SER) has been discussed²⁻⁸ rather scantily and only for the past three decades or so. SER was first noticed by Baliah and Uma² during their dipole moment measurements of some substituted anisoles. Later, Kamlet *et al.*^{3,4} invoked the same phenomenon for explaining bathochromic shifts observed in the absorption spectra of some alkyl nitrobenzenes and substituted 2,4-dinitroanilines. Similarly, the higher rates of solvolysis (4 times) shown by 3-methyl 4-methoxy benzylchloride, as compared to 4-methoxy benzylchloride have been explained in terms of the SER phenomenon⁵. Some other physical data in the literature supporting this phenomenon are: the calculated and observed dipole moment values for 2,4-dinitroanisole and 1-methoxy-2,4-dinitronaphthalene⁶, the spin-spin coupling constants between the methyl protons and the ortho ring protons of a number of substituted anisoles⁷ and the ¹⁶O and ¹³C NMR chemical shifts of 2-substituted, 2,6-disubstituted and 2,4,6-trinitroanisoles⁸. In the above-mentioned works, SER manifests in many diverse ways. The question addressed in the present communication is: can one obtain a common electronic signature for these wide-ranging physico-chemical observances regarding SER?

The recent works done by Gadre *et al.*⁹⁻¹³ have demonstrated that MESP topography is a convenient tool for monitoring the subtle changes in electronic distribution in molecules. The MESP, $V(\mathbf{r})$, at a point \mathbf{r} due to a molecular system with nuclear charges $\{Z_A\}$ located at $\{\mathbf{R}_A\}$ and electron density $\rho(\mathbf{r})$ is given by

$$V(\mathbf{r}) = \sum_A^N Z_A / |\mathbf{r} - \mathbf{R}_A| - \int \rho(\mathbf{r}') d^3\mathbf{r}' / |\mathbf{r} - \mathbf{r}'|,$$

where N is the total number of nuclei in the molecule. The terms on the rhs of the above equation represent the nuclear contribution and the electronic contributions respectively. When the electronic factor overrides the nuclear one, $V(\mathbf{r})$ becomes negative and a large negative value for it physically implies a higher electron localization around that point. These points of maximum electron localizations or minimum MESP value can be rigorously characterized by the topological analysis⁹⁻¹³ of MESP based on the identification and location of its minima.

In general SER phenomenon can occur in two ways^{4,5}. In the first case, it may occur in a system such as **1** where, X is a $+R$ or $+I$ substituent and Y and Z are $-R$ substituents⁴. It follows that progressively increasing the bulk of X forces Y from out of the ring plane, and this leads to a decrease in the power of Y to withdraw electrons from the ring and X . This is the classical situation of SHR. A counter-effect of this leads to the enhancement of the resonance interaction between X and Z and is termed as SER⁴. The second situation is observed in

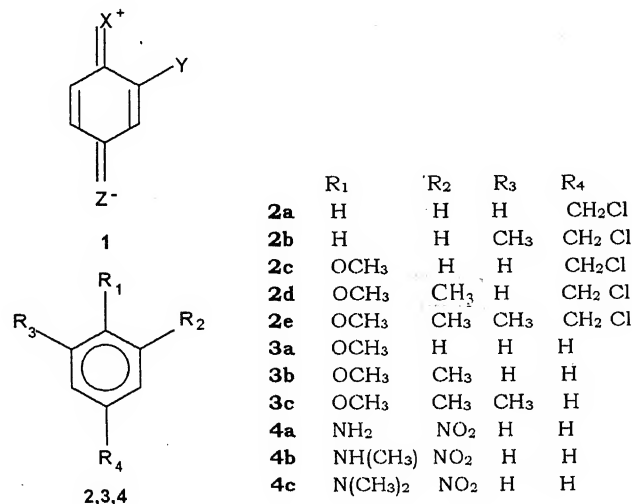


Figure 1.

substituted anisoles or thioanisoles^{2,5}. When a substituent is present ortho to the methoxyl group, the free rotation of the $-OCH_3$ group is restricted and it takes a preferred orientation that is anti to the 2-substituent and coplanar to the ring plane. This geometry enables the methoxyl group to conjugate more effectively with the group at the 4-position.

We have explored **2**, **3** and **4** (Figure 1) as test examples towards an appraisal of SER in terms of features of electron distribution. Our earlier MESP topographical analysis of substituted benzenes¹² demonstrated that the orientation effect as well as the activation or deactivation exhibited by the substituents can be very well represented by the position and the values of the MESP minimum. It was also found that using a molecular geometry optimized at a lower basis-set (typically 6-31 G) and a wave function obtained at a higher basis-set (usually 6-31 G**) is generally quite sufficient for an adequate topological representation of MESP. All the molecules reported in this work are fully optimized at HF/6-31G level using GAUSSIAN94 (ref. 14) package. Using these geometries, the wave function at HF/6-31 G** level is calculated and employed for the MESP topographical analysis. Packages UNIPROP (ref. 15) and UNIVIS (ref. 16) developed in our laboratory are employed for this purpose.

In all the substituted benzylchlorides **2a-e**, the plane which contains the chlorine, the benzyl carbon and a ring carbon is perpendicular to the ring plane. When both the ortho-positions of $-OCH_3$ are occupied by $-CH_3$ group **2e** and **3c**, the plane defined by the methoxyl carbon, oxygen and a ring carbon makes an angle 90° , which otherwise makes an angle of 0° with respect to the benzene ring. The methoxyl group and the chlorine atom are seen on the same side of the ring in

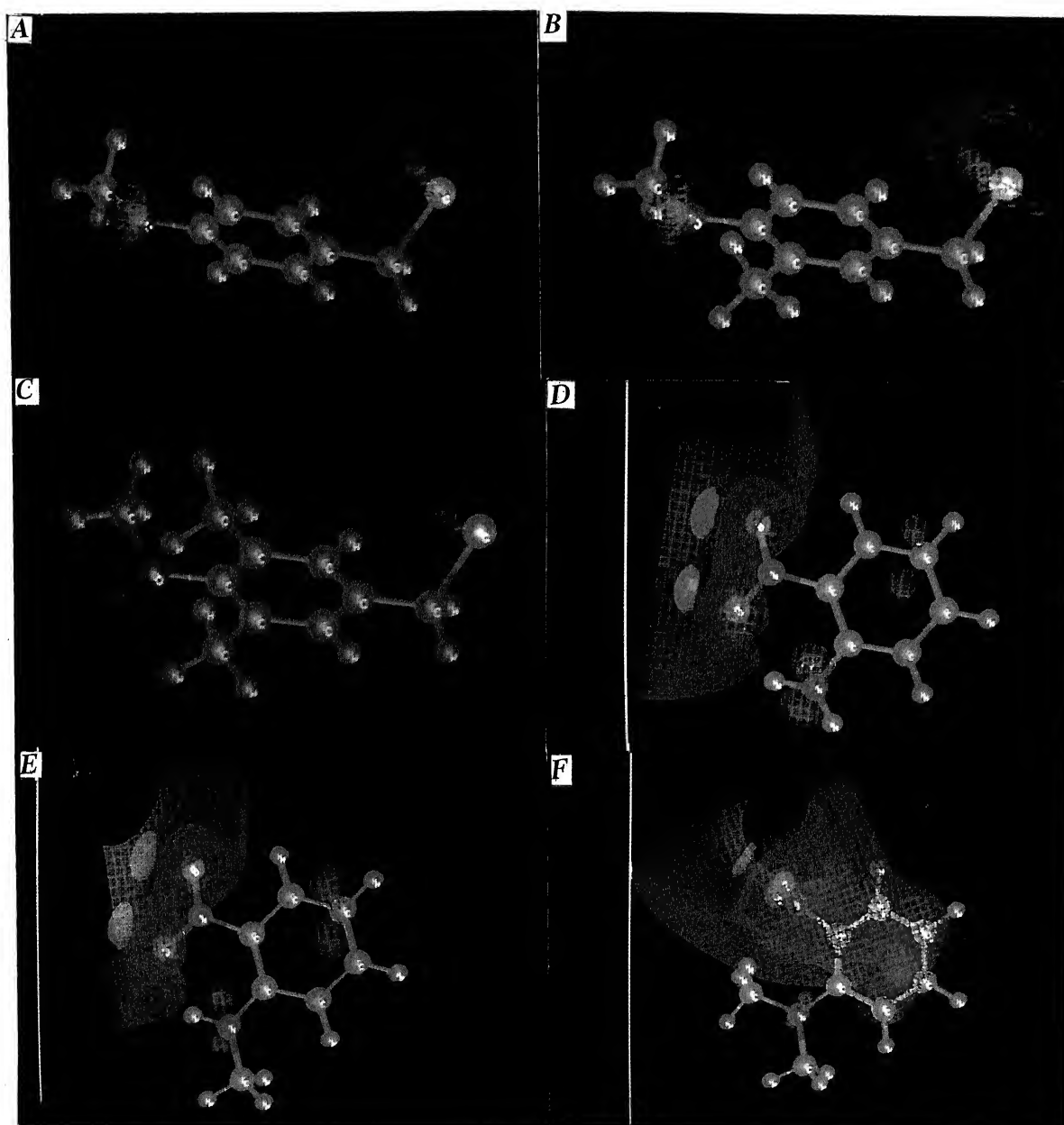


Figure 2 A–C. MESP iso-surface corresponding to $-115.41 \text{ kJ mol}^{-1}$ for systems **2c**, **2d** and **2e** respectively; D–F, MESP iso-surface corresponding to $-31.48 \text{ kJ mol}^{-1}$ (blue surfaces) and $-194.10 \text{ kJ mol}^{-1}$ (red surfaces) for systems **4a**, **4b** and **4c** respectively.

3,5-dimethyl 4-methoxybenzylchloride, **2a**. In 2-nitroanilines **4a–c**, except the case of **4c** all the heavy atoms

are lying in the benzene ring plane. In **4c**, the nitro group is not in the ring plane, but exactly perpendicular to it.

Table 1. MESP minima for systems 2, 3 and 4. All values are in kJ mol⁻¹

Structure	Oxygen minimum	Chlorine minimum	Para carbon (with respect to -OCH ₃ or -NR ₂) minimum	Nitrogen minimum
2c	-176.79	-124.07	-	-
2d	-171.28	-132.72	-	-
2e	-199.87	-125.38	-	-
3a	-200.13	-	-89.97	-
3b	-195.98	-	-96.00	-
3c	-212.46	-	-87.61	-
4a	-228.46	-	-36.20	-57.44
4b	-230.82	-	-39.61	-44.59
4c	-206.69	-	-52.46	-41.97

In benzene, the MESP minimum for the π -cloud is -85.5095 (all values quoted here are in kJmol⁻¹). The substitution of one hydrogen by the -CH₂Cl group, **2a**, deactivates the ring and the MESP value of the ring minimum is increased to -51.41. The chlorine MESP minimum is -121.71 in **2a**, which in the case of methyl chloride is -94.43, indicating that the electron density around the chlorine atom is enhanced at the expense of the ring π -electron density. The electron flow towards the chlorine is further increased when one electron donating group is introduced in the aromatic ring. A methyl group at the meta position **2b** or a methoxyl group at the para position **2c** changes the minimum ESP of chlorine to -124.07 and -129.31 respectively. When both are present, **2d**, the MESP minimum of chlorine goes down to -132.72, i.e. a decrease by 11.02 units, which is a little higher than the added effect of the -CH₃ and -OCH₃ groups. By adding one more methyl group at the remaining ortho position of -OCH₃ group, **2e**, the value of the chlorine minimum goes deeper by 3.67 than the chlorine minimum of **2a**. Exactly complementary trend in the values of the oxygen MESP minimum is observed in **2c**, **2d** and **2e** (see Table 1). Oxygen minimum is less negative in **2d** compared to **2c** and it is more negative in **2e**. A decrease in the magnitude of oxygen MESP minimum is compensated by an increase in the magnitude chlorine minimum (Table 1). This feature is graphically brought out by Figure 2, wherein iso-surface of MESP with value of -115.4116 is depicted for **2c**, **2d**, and **2e** in Figure 2A, B and C respectively. The lobe of iso-surface with value -115.41 is the largest in Figure 2C for **2e** at the expense of the chlorine lone-pair. The lobe is the largest in Figure 2B for **2d**.

The changes seen in the chlorine minimum can be correlated to the kinetic data on the solvolysis of systems **2a-e** reported by Baliah and Kanagasabapathy⁵. They have explained the unusually higher rate of reaction of system **2d** and the unusually lower reactivity of **2e** by invoking the concept of SER. In their opinion, a sub-

stituent ortho to methoxyl group does not sterically inhibit the methoxyl group from conjugating with the benzene ring, but makes the conjugation more effective than in the absence of the ortho substituent. In the present context, the observation of a highly negative valued chlorine minimum in **2d** supports their argument and it points out that such a minimum may make the leaving process of Cl⁻ easier and accounts for the higher rate of solvolysis of **2d**. When both ortho positions are occupied, **2e**, the methoxyl group prefers a perpendicular orientation with respect to the benzene ring and this makes the conjugation of the oxygen lone pairs with the benzene ring difficult, hence the -I effect of oxygen decides the electron distribution.

In the absence of the electron withdrawing group -CH₂Cl, viz. in systems **3a**, **3b** and **3c**, a MESP minimum appears above the para carbon atom with respect to the methoxyl group. A comparison of the values of the para minimum and the oxygen minimum (Table 1) once again confirms the SER phenomenon.

In 2-nitroanilines **4a-c** the interplay of the steric and the electronic effects can be monitored using three different MESP minima. Here we report one of the -NO₂ oxygen minimum, one minimum seen above the para carbon and the -NR₂ nitrogen minimum (Table 1). Going from **4a** to **4b**, the oxygen minimum as well as the carbon minimum becomes more negative (the change is 2.36 and 3.41 respectively) due to the more electron push from the -NH(CH₃) group compared to the -NH₂ group. At the same time the nitrogen minimum goes up by 12.85. When the substituent is -N(CH₃)₂ the -NO₂ group goes completely out of the ring plane and this leads to a significant increase in the oxygen MESP minimum (an increase by 21.77 with respect to **4a**), visually seen in Figure 2. This explains the classical situation of SHR. A deepening of the para carbon minimum by 16.26 shows the counter effect of this phenomenon, the SER. This effect can be visually seen in Figure 2D, E and F in terms of MESP iso-surfaces with values of -31.48 and -194.10. This enhancement is due to the more electron donation from the donor as well as the diminished electron-pulling effect of the acceptor.

The above observations on the substituted benzylchlorides and substituted anisoles lead us to the following important conclusions. There is indeed a unique electronic signature of the steric enhancement of resonance phenomenon. The phenomenon can be monitored by the electron concentration patterns through the MESP topography.

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Quantum chaos in Rydberg atoms: A quantum potential approach

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The quantum signature of chaos in Rydberg atoms has been studied using a quantum theory of motion and quantum fluid dynamics. A hydrogen atom in the electronic ground state ($n = 1$) and in an excited electronic state ($n = 20$) behaves differently when placed in an external oscillating electric field. Temporal evolutions of Shannon entropy, density correlation, phase space distance function of Bohmian trajectories and associated Kolmogorov–Sinai entropy for these two cases show marked differences.

CLASSICAL interpretation of quantum mechanics is as old as the quantum mechanics itself. In the Madelung representation¹ the time-dependent Schrödinger equation for a single particle of mass m moving under potential $V(r)$, viz.

$$[-(\hbar^2/2m)\nabla^2 + V(r)]\Psi(r, t) = i\hbar\partial\Psi/\partial t \quad (1)$$

is transformed into two-fluid dynamical equations. Substituting the following polar form of the wave function

$$\Psi(r, t) = R(r, t) \exp(iS(r, t)/\hbar) \quad (2)$$

in eq. (1) and separating the real and the imaginary parts, one obtains an equation of continuity

$$\partial\rho/\partial t + \nabla \cdot j = 0, \quad (3a)$$

and an Euler-type equation of motion

$$\partial v/\partial t + (v \cdot \nabla)v = -(1/m)\nabla(V + V_{qu}). \quad (3b)$$

In eqs (3) the charge density, $\rho(r, t)$ and current density, $j(r, t)$ are

$$\rho(r, t) = [R(r, t)]^2 \quad (4a)$$

and

$$j(r, t) = \rho(r, t)v(r, t). \quad (4b)$$

where the velocity $v(r, t)$ can be defined in terms of the phase of the wave function as

$$v(r, t) = (1/m)\nabla S(r, t). \quad (4c)$$

The quantity V_{qu} appearing in eq. (3b) is called the quantum potential or Bohm potential of hidden variable theory² and defined as

$$V_{qu} = -(\hbar^2/2m)\nabla^2 R/R. \quad (5)$$

Therefore, in this quantum fluid dynamics¹ the overall motion of the system under consideration can be thought of as the motion of a 'probability fluid' having density $\rho(r, t)$ and velocity $v(r, t)$ under the influence of the external classical potential augmented by a quantum potential, V_{qu} .

For the ground state of a many-particle system, $\rho(r, t)$ contains all information³. In a time-dependent situation

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also the time-dependent density functional theory^{4,5} asserts that any physical observable can be expressed as a functional of $\rho(r, t)$ and $j(r, t)$ and thus allows us to formulate the dynamics in terms of 'classical-like' 3D quantities. Although Madelung transformation in terms of $\rho(r, t)$ and $j(r, t)$ is not straightforward in a many-particle situation, we can make use of the time-dependent density functional theory in constructing two fluid-dynamical equations in 3D-space. This formalism is termed as quantum fluid density functional theory⁶ which has been applied in understanding ion-atom collisions⁶⁻⁸, atom-field interactions^{9,10} and electronegativity^{11,12}, hardness¹²⁻¹⁴ and entropy dynamics¹⁴ in a chemical reaction.

Quantum potential plays a crucial role in the quantum theory of motion¹⁵ as well. In this representation of quantum mechanics developed by de Broglie¹⁶ and Bohm¹⁷, the overall motion of the system is understood in terms of the motion of a particle experiencing forces originating from the classical and quantum potentials. The Newton's equation of motion for this particle guided by a wave (represented by $\Psi(r, t)$, a solution to eq. (1)) can be written as

$$(\partial/\partial t + \dot{r} \cdot \nabla)(m\dot{r}) = -\nabla(V + V_{qu})|_{r=r(t)} \quad (6)$$

At a particular instant the solution to the time dependent Schrödinger equation (1) fixes the velocity of the particle (cf. eq. 4c) and, hence, for a given initial position the particle motion can be studied through the solution $r(t)$ to the equation

$$v(r, t) = \dot{r} = (1/m)\nabla S(r, t)|_{r=r(t)} \quad (4c)$$

Theories based on quantum potential idea have been applied in solving various physico-chemical problems^{15,18-32}. Because of the presence of nonlinearity and also the 'classical language', these theories have been found^{15,21,22,30,32-34} to be helpful in understanding the quantum domain behaviour of a classically chaotic system which is described as quantum chaology by Berry³⁵. The quantum theory of motion, however, allows one to study the quantum chaos in a system without any resort to its classical domain dynamics¹⁵.

We adopt here a quantum potential approach to study the chaotic dynamics of a typical quantum system, viz. a Rydberg atom in an oscillating electric field. Of late, these systems have been considered to be 'veritable gold mines for exploring the quantum aspects of chaos'³⁶. It has also been discussed³⁷ that depending on the frequency and field intensity, hydrogen atom exhibits order to chaos transition when placed in an external oscillating field. Since hydrogen atom is one of the simplest possible solvable quantum mechanical systems and its classical mechanical counterpart, a Keplerian system, is known to exhibit chaotic dynamics³⁸ in presence of external field, we try to investigate the possible quantum signature of chaos in an electronically excited hydrogen

atom in presence of an external field. Both theoretical³⁹ and experimental⁴⁰ studies have been carried out on this system to understand the chaotic behaviour associated with quadratic Zeeman effect. For a beautiful review on this subject see ref. 36. In the present work we study the time evolution of Ψ_{1s} and Ψ_{20s} wave functions of hydrogen atom placed in an external oscillating electric field with $\omega = 5\pi$ and for both $F = 0$ and $F = 5$. We apply the quantum theory of motion in gaining insights into the possible chaotic dynamics in electronically excited hydrogen atom subjected to an external electric field^{36,37}.

The time-dependent Schrödinger equation (in a.u.) for the present problem in cylindrical polar coordinate system $(\bar{\rho}, z, \phi)$ is

$$[-\nabla^2/2 + V]\Psi = i\partial\Psi/\partial t \quad (7a)$$

where the potential V is given by

$$V = -1/r + Fz\cos\omega t. \quad (7b)$$

Making use of the following transformations

$$y = \bar{\rho}\Psi. \quad (8a)$$

$$\bar{\rho} = x^2 \quad (8b)$$

and integrating over $0 \leq \phi \leq 2\pi$, eqs (7) turn out to be

$$\begin{aligned} & [3/4x^3\partial y/\partial x - 1/4x^2\partial^2 y/\partial x^2 - \partial^2 y/\partial z^2] \\ & - [1/x^4 - 2V]y = 2i\partial y/\partial t. \end{aligned} \quad (9)$$

The above equation is solved as an initial boundary value problem using an alternating direction implicit method⁴¹. The resulting tridiagonal matrix equation is solved using Thomas algorithm. The mesh sizes adopted here are $\Delta x = \Delta z = 0.4$ a.u. and $\Delta t = 0.01$ a.u., ensuring stability of the forward-time-central-space type numerical scheme used here. Note that each alternating direction implicit cycle corresponds to $2\Delta t$ and atomic units of length and time are 0.5292×10^{-10} m and 2.4189×10^{-17} sec respectively.

The initial and boundary conditions associated with this problem are:

$$\text{at } t = 0, y(x, z) \text{ is known for } \forall x, z, \quad (10a)$$

$$y(0, z) = 0 = y(\infty, z) \quad \forall z, t, \quad (10b)$$

$$y(x, \mp \infty) = 0 \quad \forall x, t. \quad (10c)$$

The numerical scheme is stable⁴² due to the presence of $i = \sqrt{-1}$. As a further check of numerical accuracy we have verified the conservation of norm and energy (in zero field cases). The wave function is moved forward up to the end of simulation ($N\Delta t = 7$ a.u.) and then taken back to its initial position where the original profile is reproduced well within the tolerance limit of the present calculation. We could not, however, perform the long time simulation because of our inadequate computational facilities.

The dynamical quantities calculated in the present work are as follows:

- a) Shannon entropy given by

$$S = -k \int \rho \ln(\rho) dr,$$

where k is the Boltzmann constant.

- b) Density correlation function defined as

$$C = \int \rho^{1/2}(r, 0) \rho^{1/2}(r, t) dr.$$

We have also solved eq. (4c) using a second order Runge-Kutta method to generate the 'quantum trajectory' of a particle for a given initial position. Now, we are in a position to analyse the sensitive dependence on initial condition, a characteristic of a chaotic system. We can change the initial condition in two ways: i) by shifting the wave function slightly and ii) shifting the initial position of the particle slightly. In the first case the time-dependent Schrödinger equation is solved with both $\Psi(r, t=0)$ and $\Psi(r + \Delta, t=0)$ as inputs, $\Delta = 0.01$ while the second case deals with the solution of eq. (4c) with two different initial positions of the particle, (\tilde{p}, z) and $(\tilde{p} + d\tilde{p}, z + dz)$, $d\tilde{p} = 0$ $dz = 0.01$. Initial momentum of the particle is taken as zero in all cases. We study the time evolution of phase space distance (D) for the corresponding quantum trajectories defined as^{21,31,32}

$$D(t) = ((x_1(t) - x_2(t))^2 + (p_{x_1}(t) - p_{x_2}(t))^2 + (y_1(t) - y_2(t))^2 + (p_{y_1}(t) - p_{y_2}(t))^2)^{1/2}, \quad (11a)$$

where (x, p_x, y, p_y) refers to a point in phase space.

We also calculate the associated Kolmogorov-Sinai entropy as defined^{31,32} below

$$H = \sum_{\Lambda_+ > 0} \Lambda_+, \quad (11b)$$

where the Lyapunov exponent is given by^{31,32}

$$\Lambda = \lim_{t \rightarrow \infty} \frac{1}{t} \ln(D(t)/D(0)). \quad (11c)$$

'Quantum dynamics is chaotic if in a given region of phase space the flow of trajectories, according to the Hamilton-Jacobi formulation of quantum mechanics, has positive KS entropy'.³¹ The efficacy of this definition has already been tested in the cases of a quantum Henon-Heiles oscillator²¹, the quantum standard map³¹ and Weigert's quantum cat map³². For the sake of brevity unless otherwise specified, we present the time evolution of all quantities calculated for the non-zero field relative to the corresponding zero field counterpart. The Kolmogorov-Sinai entropy is calculated using this relative distance. In all plots the temporal variation is expressed in terms of the corresponding numerical integration step number, N .

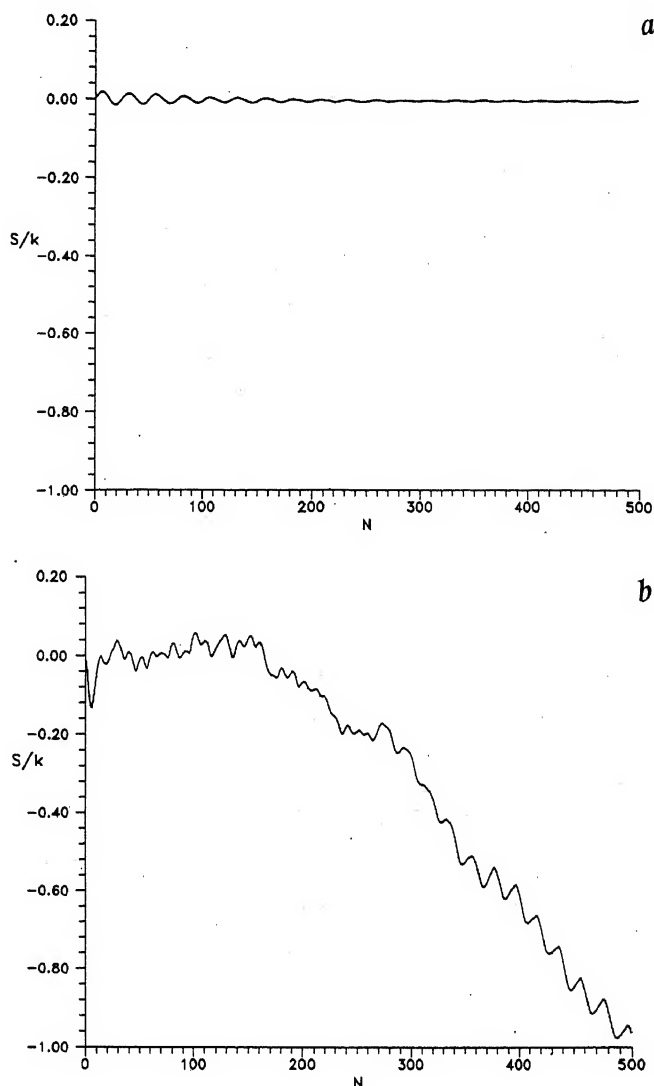


Figure 1a, b. Time evolution of S/k , where S is the Shannon entropy and k is the Boltzmann constant, for Rydberg atom in external field: (a) $n = 1$ (b) $n = 20$.

Shannon entropy and density correlation function have been shown in Figures 1a, b and 2a, b respectively. In both the figures a and b refer to the ground state and $n = 20$ state of the hydrogen atom respectively. As is clear in these figures, the applied field causes drastic change in the dynamics of Ψ_{20s} state of H-atom whereas it hardly has any effect for the ground state. In Figures 1a and 2a we see that entropy and density correlation values for $n = 1$ state do not change when the external field is applied whereas those quantities for $n = 20$ state exhibit (Figures 1b and 2b) significant changes on application of the field of same strength as in the previous case ($n = 1$).

Figures 3a, b depict the time evolution of D and H respectively for the shifted wavepacket case. For clarity we also present the behaviour of D for the non-zero field case (Figure 3c). The distance remains the same (in fact

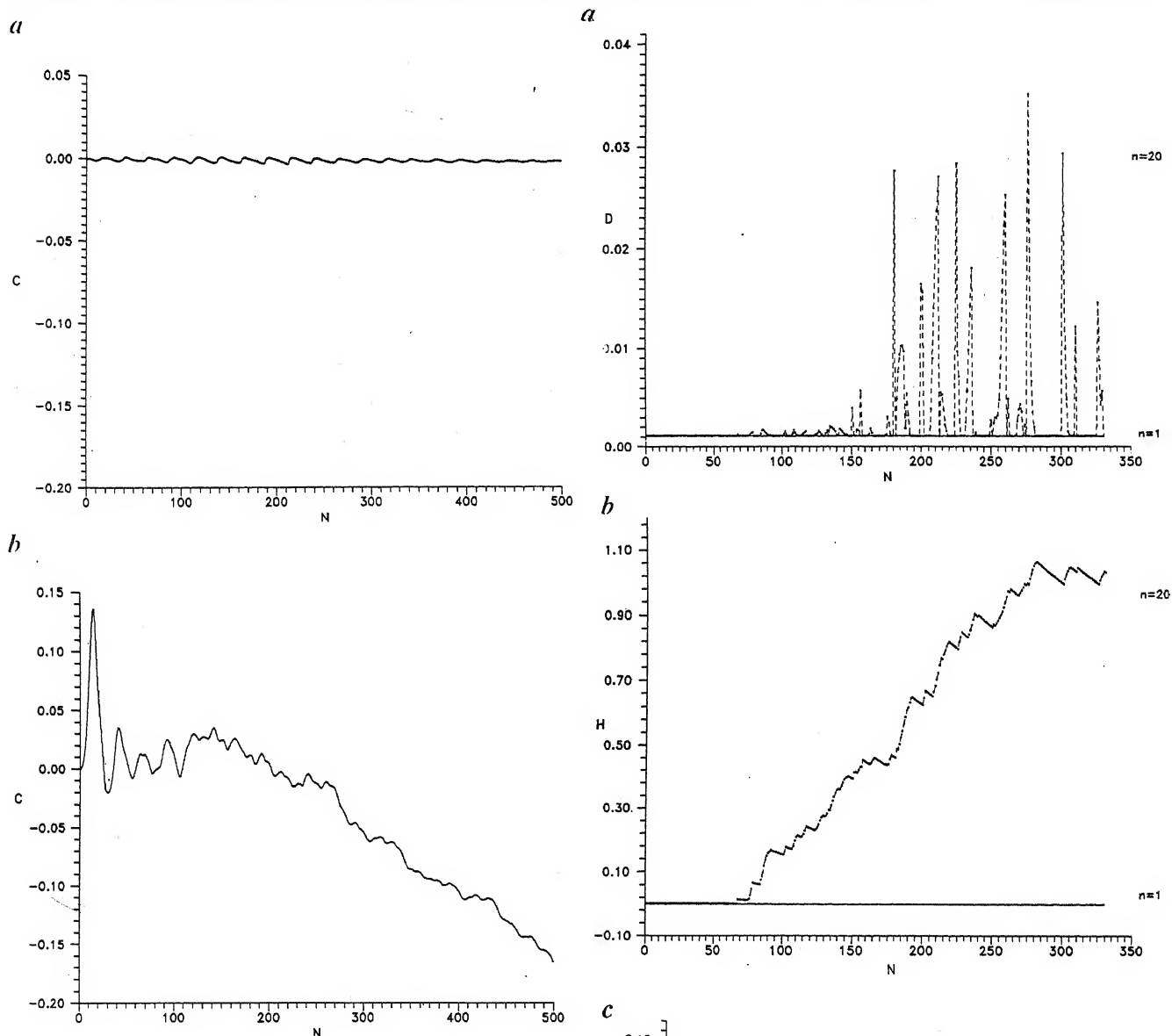


Figure 2a, b. Time evolution of density correlation function C for Rydberg atom in external field: (a) $n=1$, (b) $n=20$.

decreases at times) for $n=1$ but oscillates for $n=20$, becoming very large sometimes. Although we could simulate only up to $N\Delta t = 7$ a.u., it is clear from Figure 3b that H remains practically constant at zero value for $n=1$ while it increases rapidly to a positive value for the $n=20$ case, reflecting discernible chaotic dynamics through sensitive dependence on initial conditions. Two initially ($t=0$) nearby 'Bohmian trajectories' remain close in course of time for the $n=1$ case while they diverge for the $n=20$ case. In these figures, it is discernible that there is a time scale after which the 'chaotic' behaviour sets in. However, it needs a thorough study whether this time is related to the break-time of Chirikov and Casati³⁷. The authors are grateful to the referee for pointing this out. Corresponding D and H

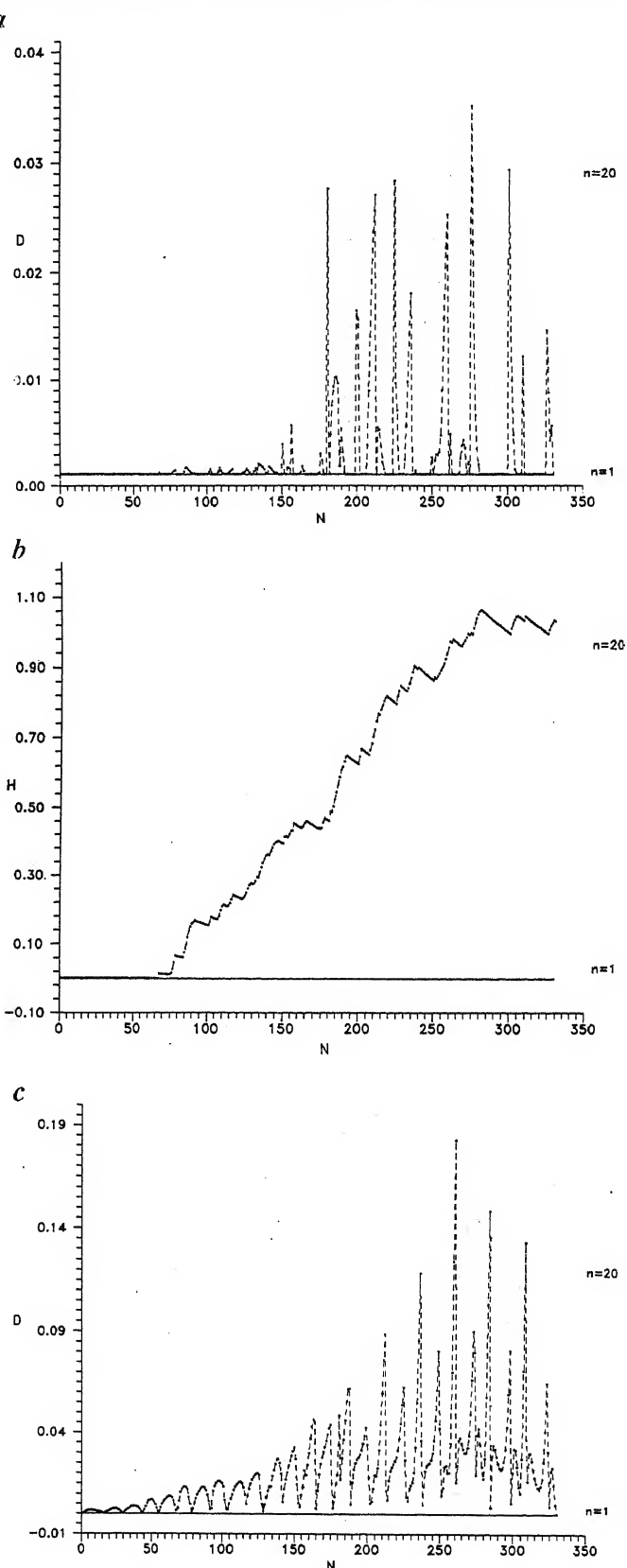


Figure 3a-c. Time evolution of phase space distance function D and KS entropy H in shifted wave function case (see text for details) for Rydberg atoms in external field: (a) D for nonzero field relative to zero field, (b) H associated with D in Figure 3a, (c) D in nonzero field.

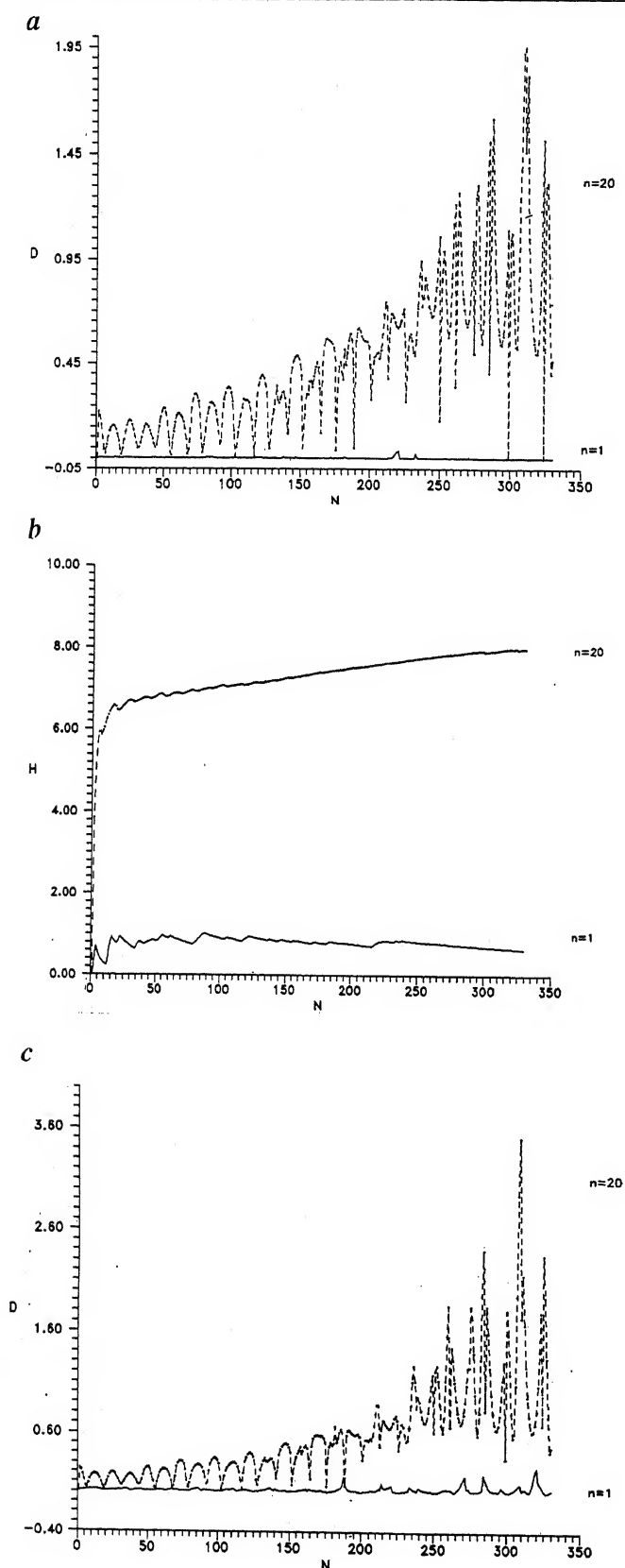


Figure 4a-c. Time evolution of phase space distance function D and KS entropy H in shifted particle position case (see text for details) for Rydberg atoms in external field: a, D for nonzero field relative to zero field; b, H associated with D in Figure 4a; c, D in nonzero field.

plots for shifted particle case are presented in Figures 4a-c. As before, Figure 4c is for the non-zero field case. Like the shifted wavepacket case, here also D remains more or less constant for $n=1$ and increases to a large value for $n=20$. The Kolmogorov-Sinai entropy initially oscillates around zero and then gradually approaches zero for $n=1$. For $n=20$ case H increases rapidly to a high positive value and then increases slowly via feeble oscillation. The negative slope in the former case *vis-à-vis* the positive slope in the latter, towards the end of the simulation, provides unmistakable signature of chaos in Rydberg atom in presence of external electric field. We have also studied (not shown here) the time evolutions of S , C , D and H for $n=5$ and $n=10$ states and also by using $F=2$. In general, for a given n , a higher value of F causes greater variations in the temporal profiles of these quantities. On the other hand, for a given F , a state with larger n value suffers more in comparison to little or no effect on a state with smaller n value. Since it requires too much of computer time we could not pinpoint the critical F value, for a given n , to exhibit the onset of chaos. In a nutshell F and n are considered to be two parameters of the present problem whose continuous variations and the associated dynamics are not studied. We have also obtained more or less similar results (not shown here) when a hydrogen atom in various electronic states experiences the time-dependent external Coulomb field due to a proton approaching for a head-on collision. It is important to note that the present short time simulation is inadequate in getting insights into the long-time asymptotic behaviour and hence one cannot infer with certainty about the final state and ionisation of the system as well as quantal suppression of chaos³⁷, if any.

In summary, we have shown that the quantum potential theories are helpful in studying the chaotic dynamics of a typical quantum system. Temporal evolution of Shannon entropy and correlation function has easily differentiated the regular and chaotic behaviour of hydrogen atom respectively in ground state and in a Rydberg state in presence of an oscillating electric field. Sensitive dependence on initial conditions is understood through time variation of phase space distance and the associated Kolmogorov-Sinai entropy. Further studies related to variations in n , F , ω , the atom, the nature of the external field, the time limit, etc. are in progress.

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Reduced uptake based zinc resistance in *Azospirillum brasilense* sp7

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Zinc resistant *Azospirillum brasilense* sp7 exhibited a low affinity for the metal. A zinc-sensitized, streptomycin-resistant variant and a sensitive mutant of the parent strain showed an increased affinity for zinc. Both the affinity for, and uptake of the metal were in the order of parent strain < sensitised variant < sensitive mutant, suggesting reduced uptake as the mechanism of resistance to zinc. Neither magnesium nor manganese could bestow any protection against zinc toxicity in the sensitive mutant, suggesting a specific pathway for the entry of the metal.

THE presence and buildup of metal pollutants in the soil affects the soil microflora, which encompass the beneficial microorganisms, like the biological nitrogen fixers or BNFs. It has been reported that the total output of BNFs could be reduced in the presence of a metal ion¹. One such nitrogen fixer is the gram negative *Azospirillum brasilense* sp7, which has gained importance owing to its non-obligate association with grasses. This bacterium expressed a high level (10 mM) tolerance to zinc (Zn) which was constitutively expressed². Continuing to work with the same strain (referred to as the parent strain hereafter), the mechanism of Zn resistance has been studied in the bacterium and is presented in this paper.

The minimal nutrient medium (MM) and culture conditions for *A. brasilense* sp7 were as mentioned earlier². A streptomycin-resistant variant, MS12, was derived with a reduced maximum tolerable concentration³ (MTC) of Zn of 2 mM. This mutation seems to be of a multifold importance in azospirilla.

A. brasilense sp7 has been known to be recalcitrant to mutagen treatments. For the members of the Azotobac-

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teriaceae, a soil treatment method⁴ has been suggested to overcome a similar difficulty⁵. The cells of the parent strain were, therefore, subjected to soil treatment to obtain a strain amenable to mutagenesis⁶ (i.e. with 5 times less DNA than the parent strain owing to the reduced size of the treated cells). The derived strain was subjected to sequential *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) mutagenesis (50 and 100 $\mu\text{g/ml}$) for 10 min in citrate buffer (pH 5.5). The mutagen was removed by centrifugation and the cell pellet suspended in normal saline was spread after appropriate dilutions. This resulted in three Zn^{S} (MTC 0.5 mM) mutants able to grow only in the presence of casamino acids. One of them, ZS2, was selected for further studies. This mutant retained the ampicillin resistance of the parent strain, and did not revert to either prototrophy or Zn resistance (frequency less than 1.6×10^{-10}).

The strains obtained were maintained as pure cultures raised from a single colony and the purity of the strains was checked periodically by testing antibiotic resistance markers, plasmid profiles, motility, cell morphology and growth on a defined medium.

In order to study the interaction of Zn with two commonly occurring divalent cations in the medium, Mg and Mn, competition experiments were conducted in the strain ZS2. 1 M stock of metal salts was prepared. Against a constant 1 mM Zn concentration, a range of Mg and Mn concentrations was tried. All the cations were added in the required amounts in the sterile MM (+0.2% casamino acids) before inoculation. Initial (0 h) and final (24 h) growth was recorded in reference to the absorbance at 560 nm. Uptake experiments were performed with ^{65}Zn (Bhabha Atomic Research Centre, Trombay, Bombay, India) at a concentration of 0.01 $\mu\text{Ci/ml}$ (0.005 $\mu\text{Ci}/\mu\text{mol}$) with a final, 2 mM Zn concentration. The assay proper was done in MM (or +casamino acids for ZS2) as described by Tynecka *et al.*⁷, except that the washing was done with MM containing 5 times Zn concentration used for assay. For determination of K_m and V_{max} , uptake was recorded after 30 min of ^{65}Zn addition, with a range of external Zn concentrations. Protein was extracted by subjecting 3 ml of culture to sonication in Vibronics Ultrasonic Processor P2 with the small probe. Three pulses of 15 sec each with an equal interval between the pulses were given. The amounts of protein were estimated by Bradford's protocol⁸ with bovine serum albumin as the standard.

No growth of ZS2 was observed in the presence of Zn with increasing concentrations of either Mg or Mn (Figure 1). Other divalent cations like cadmium, cobalt, copper, etc. could not be tested as the parent strain did not resist these metals. Also Mg^{2+} and Mn^{2+} are known to be the main competitors for Zn^{2+} in bacteria⁹⁻¹¹. However, the response of ZS2 to Zn in the presence of Mg/Mn indicated that perhaps Zn has its specific pathway, as reported for *Escherichia coli* K12 (ref. 12).

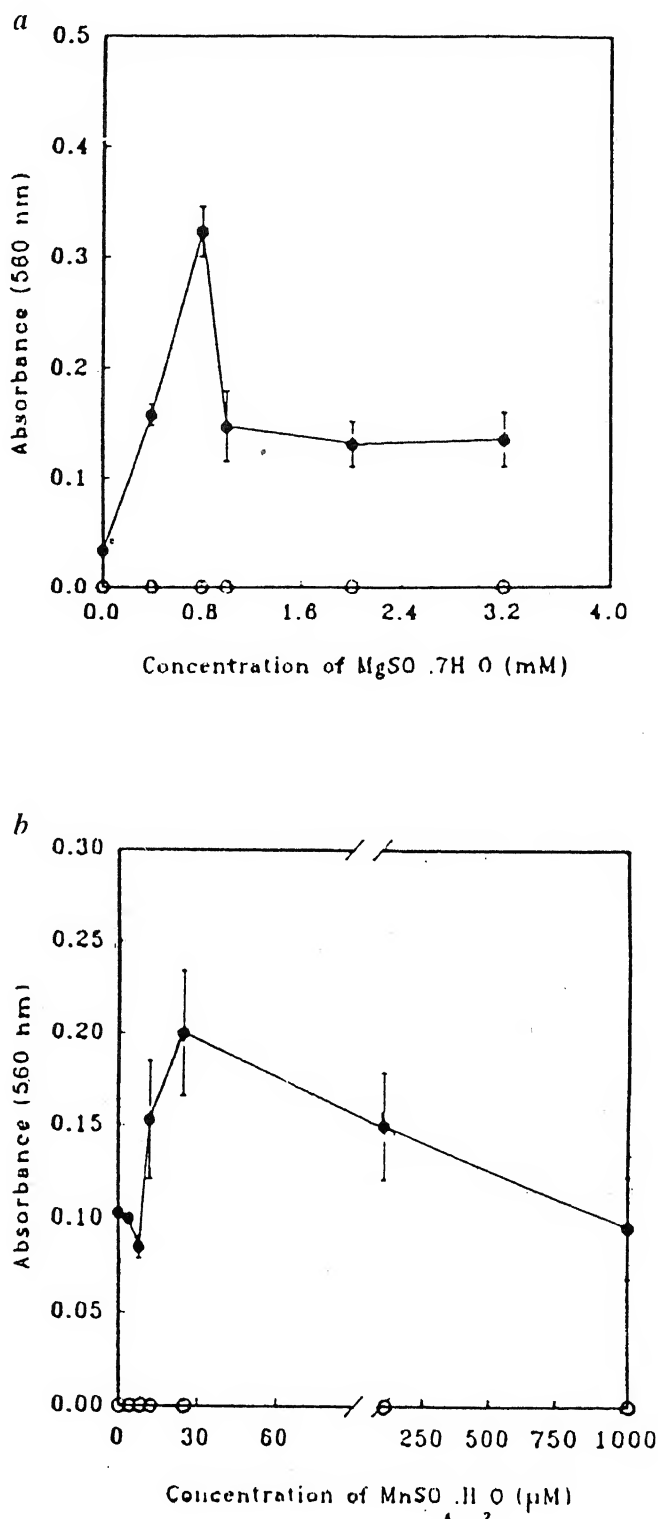


Figure 1a, b. Effect of divalent cations on the growth responses of ZS2 to Zn. Growth of ZS2 in the absence (●) and presence (○) of 1 mM Zn. Mean values \pm S.E.M. are plotted. Varying concentrations of (a) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and (b) $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ used.

The uptake pattern by *A. brasilense* sp7, variant MS12 and ZS2 is depicted in Figure 2. The behaviour of

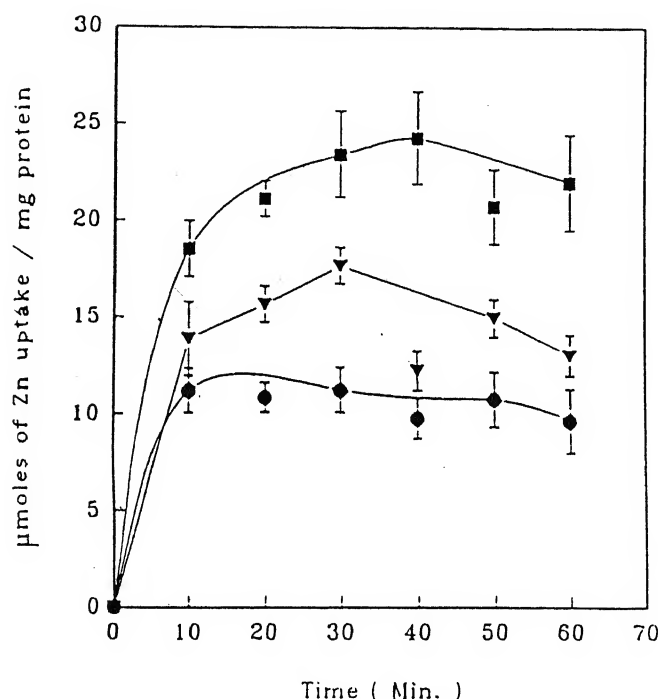


Figure 2. ⁶⁵Zn uptake in the parent strain (●), variant (▼) and the Zn^S ZS2 (■). Bars indicate ±S.E.M.

strains MS12 and ZS2 is suggestive of the reduced uptake of Zn²⁺ being the mechanism of resistance in the parent strain. The degree of sensitivity, qualitatively, was directly proportional to the amount of Zn intake. That uptake of the metal in *A. brasilense* sp7 did not involve its accumulation has been proved earlier². Reduced uptake of a metal, as the mechanism of resistance, is known in several bacterial species, e.g. for Cd²⁺ in *Staphylococcus aureus*^{7,13}, *Bacillus subtilis*¹⁴, for Cd²⁺ and Zn²⁺ in *Pseudomonas putida*¹⁵, and for CrO₄²⁻ in *P. aeruginosa*¹⁶. The response of *A. brasilense* sp7 thus conforms to this general trend in several bacteria.

While the uptake stabilized within 10 min of Zn exposure in *A. brasilense* sp7, both the variant and the sensitive mutant exhibited influx till about 30–40 min of exposure. At the maximum levels of uptake, the fold accumulation of the metal by the strain sp7, MS12 and ZS2 was found to be 3.08, 4.60 and 6.90, respectively, given the intracellular volume of water as 2.2 μl/mg proteins for *A. brasilense* sp7¹⁷.

Bacteria exhibiting obstructed or restrained uptake of the metal as the resistance mechanism show a low affinity for the metal. A consequent increased affinity in sensitive strains has been regularly observed^{7,14}. The K_m and V_{max} values calculated from Lineweaver Burk plots for all the three strains in this study followed the same

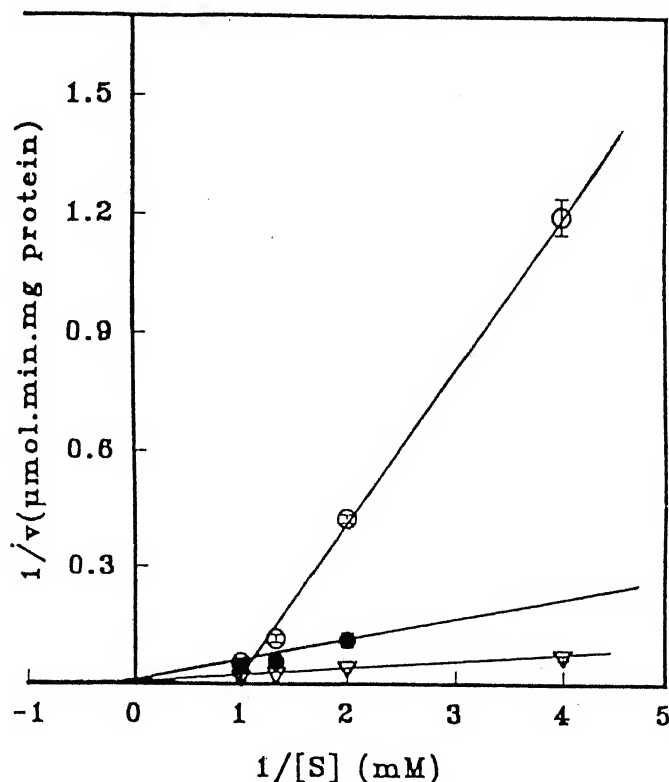


Figure 3. Double reciprocal plot for Zn uptake in strains sp7 (○), MS12 (●) and ZS2 (▼).

Table 1. The affinity for Zn²⁺ of the three strains and rates of metal uptake in *A. brasilense*

Strain	K_m (mM)	V_{max} (μmol/min/mg protein)
<i>A. brasilense</i> sp7	—	—
MS12	10.0	3.3
ZS2	2.85	13.3

pattern (Table 1) as known in other systems. The sensitive strains followed the Michaelis-Menten saturation kinetics, while the Zn-resistant parent strain failed to exhibit the saturation, similar to the observation in *B. subtilis*^{14,18}. The reciprocal plot constructed for the latter was, thus, unusual (Figure 3). We have concluded that *A. brasilense* sp7 exhibited a lack of affinity for Zn and this results in rendering the organism resistant. We assign this lack of affinity towards Zn²⁺ due to a typical response exhibited by sp7 cells in the presence of the metal. As reported by us earlier, the cells get enlarged, show an increased level of exopolysaccharides over control and release a melanin-like pigment³.

Though the data on metal resistance in azospirilla are available, they are preliminary and sparse^{19,20}. In this

investigation, efforts have been made to unravel the mechanism of resistance to Zn in this hitherto unexplored organism as a step towards effective biofertilizer application. This study also fulfils the need to understand the effect of arbitrary application of metal-containing pesticides on numerous non-target microorganisms. The efficacy of Zn^r *A. brasilense* when applied to maize seeds in *in vitro* studies has already been established in our laboratory²¹.

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Ammonia volatilization and denitrification losses from nitrogen fertilized flooded soil as affected by the addition of iron pyrites

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Results of a laboratory study indicated that iron pyrites reduced nitrous oxide (N₂O) and NH₃ evolution from flooded soil fertilized with KNO₃ or urea. Total gaseous (N₂O + NH₃) loss was reduced by 47.3% with the addition of pyrite compared to the urea alone treatment.

GASEOUS N loss through NH₃ volatilization and denitrification is responsible for the low N use efficiency of rice under flooded conditions¹. Nitrification² and urease inhibitors³ and slow-release fertilizers⁴ are proposed as strategies to reduce N loss and enhance NUE. Nitrification inhibitors reduce losses through denitrification^{5,6} but have been found to accentuate NH₃ loss⁷. Iron pyrites, a mineral occurring in plenty as sedimentary deposits in parts of Bihar, India, have been found to inhibit

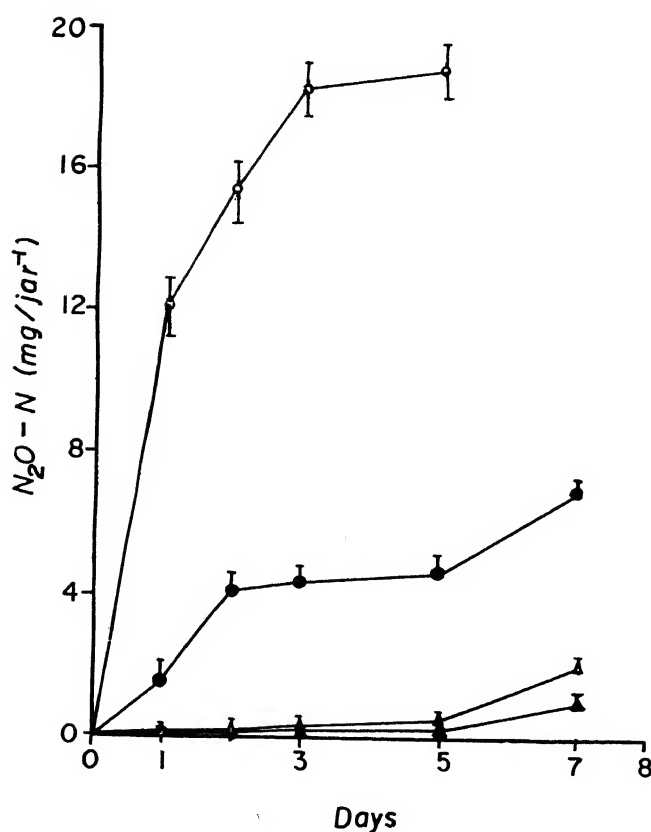
nitrification⁸ and reduce NH₃ loss from surface-applied urea⁹. Since pyrites retard nitrification, it could possibly reduce denitrification. Hence, an incubation study was conducted in the laboratory to find out the effect of pyrite on denitrification and NH₃ volatilization from urea applied to a flooded soil.

Surface soil (0–15 cm) from cultivated fields of Duerast, Freising, Germany, having pH 6.8 (soil:CaCl₂ 1:2.5), organic C 1.12%, 4.1 mg NH₄⁺-N and 15.6 mg NO₃⁻-N kg⁻¹ soil, total N 0.12% was used in the study. Air-dry soil (200 g) was placed in 500 ml jars. The soil was flooded to a depth of 2 cm and pre-incubated for two weeks. To enhance the reduced conditions, glucose solution equivalent to 500 mg C kg⁻¹ soil was applied. The mineral-N status of soil after pre-incubation was 6.3 mg NH₄⁺-N and 0.1 mg NO₃⁻-N kg⁻¹ soil. After the pre-incubation, the soils were treated with N at the rate of 100 mg kg⁻¹ soil in the form of KNO₃ and urea solution, separately, with or without pyrite. Pyrites, obtained from Pyrites and Phosphates Chemicals Limited (PPCL) New Delhi, was added equivalent to 100 mg S kg⁻¹ soil. Each treatment combination was replicated thrice. A no N control was also maintained. The jars were covered with a lid provided with a rubber septum. The sides of the lid and rubber septum were sealed with silicone to prevent gas leaks. After 1, 2, 3, 5 and 7 days, gas samples were taken and analysed for N₂O-N by gas chromatography (Varian 3400) using an electron capture

Table 1. Effect of pyrite on NH_3 loss from urea applied to a flooded soil ($20 \text{ mg urea-N } 200 \text{ g soil}^{-1} \text{ jar}^{-1}$)

	Days			Total
	1	2	7	
	(mg $\text{NH}_3\text{-N jar}^{-1}$)			
Urea	$0.62 \pm 0.078^*$	0.46 ± 0.024	0.41 ± 0.019	1.49 ± 0.104
Urea + pyrite	0.53 ± 0.016	0.36 ± 0.046	0.054 ± 0.0034	0.94 ± 0.062

*Standard error.

**Figure 1.** Effect of iron pyrite on nitrous oxide evolution from flooded soil (O, KNO_3 ; ●, KNO_3 + pyrite; Δ, urea; ▲, urea + pyrite). Vertical bars indicate standard error; LSD ($P < 0.01$) = 0.73 for day seven.

detector. For estimating NH_3 loss, a separate set of jars of each treatment in triplicate was set up similar to the previous one. The jars in this case were covered with parafilm provided with a few micropores for aeration. The NH_3 evolved was trapped in 4% 10 ml boric acid-mixed indicator solution contained in vials placed at the soil surface⁹. The trapped NH_3 was estimated by titrating with H_2SO_4 (0.005 N).

By day 5, almost the entire applied N was recovered as $\text{N}_2\text{O-N}$ with KNO_3 -treated soils (Figure 1). Addition of pyrite resulted in lower N_2O evolution. At the end of 7 days, amount of $\text{N}_2\text{O-N}$ recovered was 34.5% of ap-

plied N with KNO_3 + pyrite treated soils compared to KNO_3 alone treatment. Under reduced conditions, nitrate is known to be rapidly denitrified¹⁰. On the other hand, urea-treated soils had low N_2O evolution. Most loss occurred between fifth and seventh day. The slow urea hydrolysis¹¹ and nitrification^{12,13} processes under flooded conditions are responsible for the initial lag period observed with the urea-treated soils. Urea + pyrite-treated soils had less than half the N_2O evolution compared to urea alone treatment. This could be due to the nitrification inhibitory effect of pyrite⁸. But we observed that the inhibitory effect of pyrite is rather very low in flooded soils. The most plausible reason appears to be that pyrite is directly involved in inhibiting nitrite- or nitric- or $\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$ nitrous oxide reductases. This is very much evident from the low N_2O evolution observed with KNO_3 + pyrite treatment. Kowalenko¹⁴ reported S anions to reduce denitrification loss, which suggests that the sulphides in iron pyrites could play a major role in the process. The other possible explanation could be that as NO_3^- becomes limiting, N_2O is used as an electron source reducing it to N_2 (ref. 15).

Ammonia volatilization was observed only in the urea-treated soils (Table 1). NH_3 evolution was observed right from day one, which declined later possibly due to it being nitrified or adsorption by the soil¹³. Addition of pyrite reduced NH_3 loss from urea by 37%. This is attributed to the acidic nature of pyrite¹⁶ preventing the rise in floodwater pH⁹. Total gaseous-N ($\text{N}_2\text{O} + \text{NH}_3$) loss was 3.49 mg jar^{-1} for the urea alone treatment which was brought down to 1.85 mg jar^{-1} with the addition of pyrite, a reduction of 47.3%. The results also indicate the NH_3 volatilization and denitrification losses to be interdependent¹⁷. The possibility of losses beyond 7 days cannot be eliminated. However, earlier studies^{18,19} showed maximum loss to occur within first 10 days.

From the results of this study it is not clear whether pyrite reduces total denitrification as the other NO_x components could not be analysed. The effect of pyrite on denitrification, therefore, needs to be probed further. However, it does offer a benefit by way of reducing NH_3 loss and N_2O evolution which is implicated in the depletion of stratospheric ozone layer.

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Biological control of blast disease of fingermillet (*Eleusine coracana* L.) and an analysis of fertility of *Magnaporthe grisea*

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Six strains of fluorescent pseudomonads which belong to *Pseudomonas fluorescens* and *P. putida* were screened in the laboratory for their ability to inhibit the ragi blast fungus *P. grisea*. The bacterial strains showed fungal inhibition in dual plate assays in the laboratory and reduced ragi blast severity in the field. Leaf and neck blast were reduced by 45.3 to 65.2% and 24.3 to 54.9% in moderately resistant ragi cv. CO-7 and by 59.1 to 72% and 33.3 to 63.3% respectively in the susceptible cv. PR-202. *P. fluorescens* strains 7–14 was most effective amongst the bacterial treatments. For the first time, perithecial formation occurred in 11 of the 96 *P. grisea* combinations when they were mated in the laboratory with known testers of rice blast fungus which were used as a parent.

FINGERMILLET (*Eleusine coracana* L.) commonly known as ragi ranks second in importance among the millets in India and is widely cultivated in several parts of Tamil Nadu, Andhra Pradesh, Karnataka and Maharashtra. Blast incited by *Pyricularia grisea* (Cke) Sacc, is one of the major destructive diseases causing excessive damage to this crop from seedling to earhead-forming stages.

The disease occurs during all growing seasons and on almost all ragi varieties cultivated.

Considering the hazards of chemical applications, biological control has emerged as an important alternate strategy for disease control in recent years. There has been much success in biological control of crop diseases by using antagonistic bacteria of the fluorescent pseudomonad group^{1–3}. Studies have revealed the potential of antagonistic microorganisms in inhibiting pathogens at the root–soil interface, thereby protecting perennial and annual plants such as cotton, potato, tobacco, wheat and rice^{4–7}. Thomoshow and Weller⁸, Weller and Cook⁹ and Schippers *et al.*¹⁰ found these microorganisms to inhibit pathogens by producing antibiotics, siderophores (compounds that chelate biologically available iron) and plant growth-stimulating substances. Earlier studies from our laboratory have shown that, when carefully selected strains of *Pseudomonas fluorescens* or *P. putida* were used as bacterial treatments, such treatments resulted in the reduction of blast, sheath-rot and sheath blight severities in rice^{11–15}.

The discovery of the sexual stage¹⁶ in *P. grisea* has broadened the understanding of genetic variation in the genus *Pyricularia* and is important for effective blast disease management, as resistant breeding strategies are based on the clonality of the pathogen. Complexity of fertility in this fungus is partially responsible for incompatibility amongst isolates. The purpose of the present fertility studies is to determine the sexual compatibility of the Indian isolates of *P. grisea* when mated with fertile testers. We have evaluated a selected set of fluorescent pseudomonads for the suppression of ragi blast in the laboratory and in the field.

Strains of fluorescent pseudomonads, *P. fluorescens* Pf KE26, Pf TNC82, Pf 7-14, Pf Pfcp and *P. putida* Pp U113b, and Pp V14i isolated in our laboratory were used in the present study. Infected finger millet tissues were collected from the South Indian states of Tamil Nadu, Andhra Pradesh, Kerala and Karnataka from 1993 to 1995 and 96 monoconidials of *P. grisea* were isolated.

Crosses between 24 ragi isolates and four rice isolates (testers), GY-11, KA-3, KA-7 and KA-9 (obtained as gift from J. L. Notteghem, CIRAD, Montpellier, France) were made by placing mycelial agar blocks of the paired isolates about 5 cm apart from each other on oatmeal agar plates. The petri dishes were incubated at 27°C for about a week and then transferred to 22–24°C under continuous illumination. Fertility of these strains was monitored by observations on perithecial formation.

For *in vitro* assay of antibiosis, 10 ml of sterile water was poured into slant cultures of *P. grisea* and the surface of mycelial growth scraped. This suspension was spread on PDA plates and air-dried. Fresh bacterial cell suspensions that contained 10^9 colony-forming units/ml were prepared from 24 h grown bacterial cultures raised on Kings' B medium and were spotted at the centre of the plates using sterile toothpicks. Plates without bacterial inoculation served as control. Antifungal activity was determined by measuring the diameter of inhibition zones. Three replications were maintained for each bacterial strain evaluated.

Two field experiments and one seedbed experiment were conducted at three different locations during 1993 to 1995. The field experiments were conducted at Anna University, Madras and at Pallikaranai, a suburb of Madras. The seedbed experiment was made at the Regional Agricultural Research Station, Pattambi, Kerala which is a hot-spot for the disease. Ragi seeds (CO-7 and PR-202) obtained from the University of Agricultural Sciences, Hebbal, Bangalore were used for the study. Seeds for the field experiments were sown in a randomized complete block design (RCBD) with three replications in field plots of 1.5 × 1.0 m. The fungicide tricyclazole (beam) was also included as a treatment for making comparisons. Two most efficient strains that afforded maximum disease control in the first experiment were selected and evaluated in the seedbed experiment. For the seedbed experiment, seeds were sown in 1 m wide rows and were maintained in upland condition.

Bacteria were grown in King's B medium broth for 24 h and the cultures were adjusted to an OD of 0.1 at 600 nm with 1% w/v carboxymethylcellulose (cmc) to obtain a cell concentration of 10^9 cfu/ml. Surface-sterilized seeds were soaked in the bacterial suspension for 12 h at 28°C and air-dried. At the time of sowing, seeds had a 10^9 cfu (colony forming units) per gram. Likewise, seeds were treated with the fungicide at the

concentration of 2 g a.i./kg seed. Seeds treated with sterile cmc served as check. Besides seed treatment, plants were given foliar sprays with the bacteria or fungicide on 15, 25 and 35 days after sowing. Fungicide was sprayed at a concentration of 400 g a.i./ha. Plants were inoculated with the blast pathogen on the 36th day. For artificial induction of blast, *P. grisea* inoculum was raised on a medium that consisted of sterilized grass leaves (15 g) and sucrose (2 g). After 15 to 20 days, the spores were dislodged and the spore concentration was adjusted to 50,000 conidia/ml in 0.5% gelatin solution according to the method of Mackill and Bonman¹⁷ and sprayed. Disease was scored by random examination of 15 plants per plot for leaf blast and 25 plants for neck blast. Disease was scored in the 0 to 5 scale developed by the All India Co-ordinated Small Millets Improvement Project. Leaf blast severity index (LBSI) and percent disease reduction were calculated and the data subjected to statistical analysis.

Perithecial formation was observed in 11 of the 96 crosses made. Mature perithecia were formed at the intersection of growth of the fertile and compatible isolates after 28–32 days. They occurred singly or in groups and were partially embedded in the medium with long beaks protruding from the agar surface. The release of asci from a mature perithecium is shown in Figure 1.

All the six bacterial strains inhibited the growth of *P. grisea* *in vitro*. The bacterial strains differed in their ability to inhibit the different isolates of *P. grisea* and induced inhibition zones varying from 10 to 33 mm in

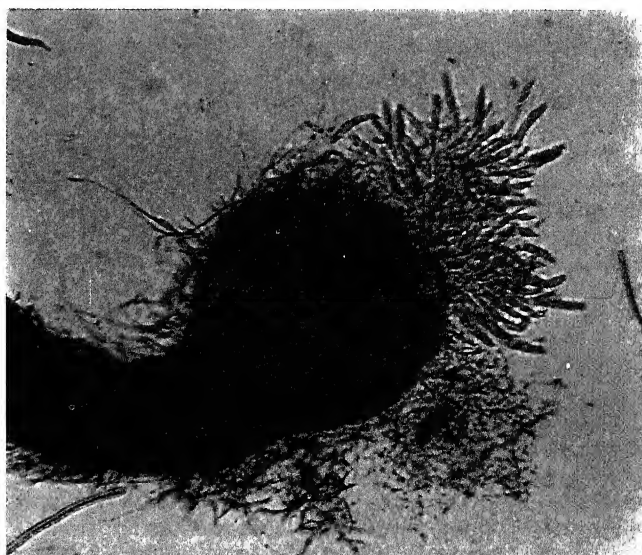


Figure 1. Release of asci from a mature perithecium produced in the laboratory. Mature perithecia were formed by *P. grisea* isolates pathogenic to finger millet when they were mated with known tester isolates of *P. grisea* pathogenic to rice. The testers were obtained from J. L. Notteghem, France²².

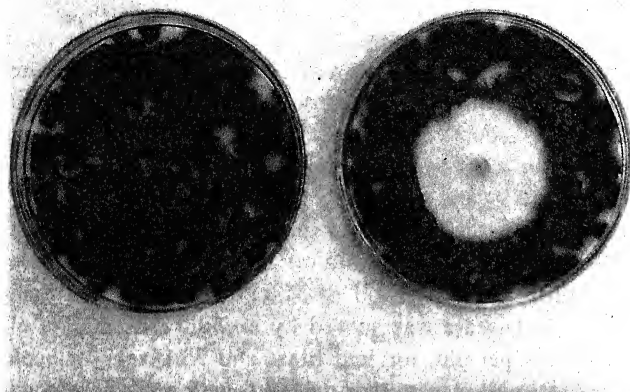


Figure 2. Dual plate assay that shows the inhibition of *Pyricularia grisea* by *Pseudomonas fluorescens* Pf 7-14 on a PDA plate. Such assays are used in the laboratory for locating efficient antagonists.

Table 1. Reduction of finger millet blast in cv. CO-7 (Experiment 1) and cv. PR-202 (Experiment 2) by bacterial treatments, Madras, 1994, 1995

Treatment	Experiment 1 cv. CO-7 Percent control		Experiment 2 cv. PR-202 Percent control	
	Leaf blast	Neck blast	Leaf blast	Neck blast
Pf KE-26	49.0 ^a	42.9	67.6	33.3
Pf TNC-82	56.3	38.6	60.4	41.1
Pf 7-14	65.2	54.9	72.1	63.3
Pf Pfcp	64.4	52.9	70.3	52.2
Pf V14i	54.8	42.9	69.6	52.2
Pp U113b	45.3	24.3	59.1	33.3
Tricyclazole	68.2	57.1	74.1	66.6
Control	0.0	0.0	0.0	0.0
	LSD (0.05) = 6.37 (0.01) = 8.84		LSD (0.05) = 5.03 (0.01) = 7.06	

^aEach figure is a mean of three replications.

Table 2. Effect of bacterial treatments on the yield components of ragi cv. PR-202, Pallikaranai experiment, 1995

Treatment	Mean seeds per finger	No. of fingers examined	1000 grain weight		Total yield (g)
			Fresh wt(g)	Dry wt (g)	
Pf KE-26	355	10	2.23	1.89	320
Pf TNC-82	326	10	2.26	1.88	325
Pf 7-14	371	10	2.41	2.02	402
Pf Pfcp	368	10	2.40	2.00	395
Pp V14i	342	10	2.27	1.90	362
Pp U113b	345	10	2.23	1.82	360
Tricyclazole	376	10	2.35	1.97	442
Control	350	10	2.10	1.78	290
			LSD $r = 0.67$ (0.05) = 13.2 (0.01) = 18.3		



Figure 3. Biological suppression of finger millet of blast by *Pseudomonas fluorescens* Pf 7-14 in cv. PR-202 plants. Bacteria were applied as seed treatment and as three additional foliar sprays. Leaf blast was induced by artificial inoculation of *Pyricularia grisea*. Finger millet leaves on the right are from bacterial treatment and they show substantial reduction of leaf blast. On the left are finger millet leaves from untreated control plants.

diameter. Strains Pf KE-26 and Pf 7-14 afforded maximum inhibition zones of 30 and 33 mm diameter against *P. grisea* isolate 21.1.2 (Figure 2). Strains Pf TNC-82, Pf Pfcp and Pp v14i showed maximum inhibition against isolate 19.1.1 (24, 27.5 and 19 mm) respectively.

Data on suppression of blast afforded by the bacterial strains in the field tests are given in Tables 1 and 2. In field experiment 1, leaf blast control ranged from 45.3 to 65.2% and neck blast from 24.3 to 54.9% with bacterial treatments while in the second, 59.1 to 72% reduction in leaf blast and 33.3 to 63.3% reduction in neck blast were observed (Table 1). The fungicide tricyclazole proved superior in controlling blast in both the varieties tested. Figure 3 shows the substantial reduction of leaf blast afforded by Pf 7-14. There was a significant increase in total grain yield and 1000 grain weight due to bacterial treatments (Table 2). When two of the most efficient *P. fluorescens* strains Pf 7-14 and Pf Pfcp were evaluated further in seedbed at Pattambi, Kerala, these strains reduced leaf blast by 69.1% and 63.2% while the systemic fungicide reduced leaf blast by 72.9% (Table 3). There was no significant increase in total grain yield.

Table 3. Effect of bacterial treatments with *P. fluorescens* strains (Pf 7-14 and Pf Pfcp) on leaf blast reduction and yield in ragi cv. PR-202 seedbed experiment, Pattambi, Kerala

Treatment	Leaf blast		Grain yield (g)*
	Percent incidence	Percent control	
Pf Pfcp	27.6	63.2	325
Pf 7-14	23.2	69.1	355
Tricyclazole	20.3	72.9	350
Control	74.9	0.0	300
LSD			
(0.05) = 4.2			
(0.01) = 6.4			

*LSD analysis not significant.

Our results from *in vitro* studies, field trails and seedbed experiment show the efficacy of fluorescent pseudomonads in suppressing the growth of *P. grisea* and the development of ragi blast. The difference in the zones of inhibition with the various pathogen isolates could be due to the variance in virulence amongst *P. grisea* isolates. Fravel¹⁸ observed that the parameters affecting the production of secondary metabolites could modify *in vitro* antibiosis. There was good correlation between *in vitro* antibiosis and disease reduction. Similar results have been reported earlier¹⁹⁻²¹. Amongst the bacterial treatments, strain of 7-14, a proven biocontrol agent afforded most leaf and neck blast control. Though disease control afforded by bacteria was not equivalent to that afforded by tricyclazole, there was a significant reduction in disease with the bacterial treatments (Table 1).

The potential of *Pseudomonas* spp in suppressing rice blast when applied as seed bacterization and as additional foliar sprays has been previously demonstrated¹⁴. In the study conducted by Gnanamanickam *et al.*¹² at the International Rice Research Institute (IRRI), Philippines, it was shown that fluorescent pseudomonads reduced rice blast severity when applied as seed treatment and foliar sprays. Several reports have suggested that antibiotics produced by *P. fluorescens* strains play an important role in disease suppression^{5,8,11,14,18}.

Formation of perithecia by *P. grisea* isolates pathogenic to ragi has not been observed in India. The sexual stage usually does not occur in the field. With the help of known tester isolates²², we have been able to observe the sexual stage of the blast fungus, *Magnaporthe grisea* (Figure 1). With the help of this genetic tool, it is possible to study the genetics of pathogenicity or virulence of the Indian isolates of *M. grisea* towards ragi and rice.

From this study it is clear that bacterial strains can be efficiently used to control blast when used as seed treatment and foliar sprays. The results of bacterization

with fluorescent pseudomonads are encouraging. The present study suggests that carefully selected strains *P. fluorescens*/*P. putida* can be developed as biological formulations for field-level application by our resource-poor farmers for effective management of ragi blast. With the observation that the ragi strains are fertile and produce the sexual stage, *M. grisea* in laboratory crosses with known testers of rice, the genetics of virulence in *M. grisea* towards ragi and rice can be established and we are pursuing this at present.

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Discovery and invention

Sir Martin Rees

In Victorian times, the national scientific enterprise was minuscule by today's standards. Yet the commitment to the public understanding of science was not. The marvellous national and civic museums – cathedrals of discovery and invention – consumed large resources by the standards of that time. Our forebears believed, as fervently as the British Association does now, that the UK's achievements in science, engineering, and technology deserve wider appreciation, that science is part of our culture, and that how it is applied should concern us all.

The 'public' for science

Science and engineering had a high profile. Most people have heard of the great nineteenth century engineers – Brunel, Telford, and so forth. It is actually harder to name living engineers even though their marvels surpass those of earlier centuries. And it was not just the practical men – the 'wealth creators' – who earned public acclaim. Think of Darwin: his insights had no practical payoff, but he was a revered figure because he changed the way humans see their place in nature.

So what about public attitudes today, as we approach the twenty-first century? We have all read the outcome of scientific quizzes, in newspapers and elsewhere, which reveal that many can hardly tell a proton from a protein. Such people can (partially) excuse themselves by claiming that the facts in themselves are not the essence. What matters is having a rough 'intellectual map': so that we can appreciate our natural environment; so that the artefacts that surround us do not seem mysterious; and so that we can all participate, critically, in shaping how technologies are developed and applied.

Everyone needs a basis for assessing when scientific claims are credible and when they are not. Noisy controversy does not always signify evenly balanced arguments; but most issues that rightly concern us involve genuine scientific uncertainties, and major trade offs. The ethical and social implications of (for instance) *in vitro* fertilisation from foetuses, genetic screening, or environmental degradation can and should be widely appreciated and discussed, even by people who do not understand (and may not be specially interested in) the science *per se*.

The adult public is very heterogeneous. All of us are part of it. Professional scientists are depressingly 'lay' outside their specialisms – I depend on 'popular' presentations in the media for updates on biomedical topics. Broadcasts or newspaper articles about my own professional interests deepen my respect for journalists who successfully cover all the sciences, working to tight deadlines. I know, from experience, how hard it is to explain non-technically, even something in my specialist field.

Science generally earns a newspaper headline, or a place on the TV bulletins, only as a background rather than as a story in its own right. Indeed, coverage restricted to 'newsworthy' items – newly announced results that carry a crisp and easily summarisable message – cannot avoid distorting how science develops. Scientists cannot reasonably complain about this any more than novelists or composers would complain that their new works do not make news bulletins. The place of science is in features, documentaries, and so forth rather than news.

A recent *Daily Telegraph* poll asked people on what topics they would like to see more newspaper coverage. Top choice was medicine; science and invention tied with crime for second place.

Within science, it is often the utterly 'irrelevant' subjects that fascinate people most. Dinosaurs have topped the charts since Richard Owen announced their discovery at the British Association

in 1841. Cosmology runs dinosaurs close; so does human origins. Despite an intellectual climate where some scientific advances arouse unease, these subjects have retained a positive and non-threatening image.

Communicating science: a researcher's perspective

Researchers do not usually shoot directly for a grand goal. Unless they are geniuses (or unless they are cranks) they focus on 'bite sized' problems that seem timely and tractable. That is the methodology that pays off. However, it carries an occupational risk – we may forget that we are wearing blinkers and that our piecemeal efforts are only worthwhile insofar as they are steps towards some fundamental question. The physicist Arno Penzias, who made a really great discovery, said that he did not himself appreciate its full significance until he read a 'popular' description of it in the *New York Times*. Presenting our work as clearly as we can to general audiences, who do not care about the details, helps us to see it in perspective. (Niels Bohr said that you should speak as clearly as you think, but no more so. That is a good maxim – though Bohr himself apparently took caution to excess by mumbling inaudibly and incomprehensibly!)

Another salutary question for scientists is this: if you could inject one idea from your subject into 'common culture', what would it be? I should choose cosmic evolution.

At British Association meetings in the 1860s, Darwin's ideas were boisterously debated; they were part of the nineteenth century culture. He showed (to quote the famous final words of the 'Origin of species') how, 'whilst this planet has been cycling on according to the fixed law of gravity, from so simple a beginning, forms most wonderful... have been, and are being, evolved'. Cosmologists now go back before Darwin's 'simple beginning'. They view our entire solar system in a broad evo-

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lutionary context, stretching back to when the Milky Way galaxy first formed – right back, even to the hot dense fireball from which our entire universe emerged.

When a star explodes as a supernova, astronomers and physicists study it eagerly. Yet why should anyone else be interested in exploding stars thousands of light years away? The answer is that they hold the answer to the question 'where did the atoms we are made of come from?' Were it not for supernovae, none of us (nor even the earth) could exist.

Stars and nebulae contain roughly the same proportions of the different chemical elements as our solar system. What determines this universal mix? Did a creator turn 92 different knobs? The answer is no. Atoms on earth were forged by nuclear fusion in ancient stars that exploded as supernovae somewhere in the Milky Way. The atoms then found themselves in an interstellar cloud that condensed into new stars, some with retinues of planets. One of these stars was our sun. We can calculate the resultant 'mix' – why carbon and oxygen are common, but gold and uranium are rare. Galaxies are like vast ecosystems, in which pristine hydrogen is recycled through successive generations of stars, gradually building up the entire periodic table.

That link between us and the stars is just an example of the kind of concept that I should like to be part of the broader culture. It stimulates, of course, another question: where did the hydrogen itself come from? The answer itself lies in the initial big bang which set our entire universe expanding – another (more uncertain) story. However, it leads into my next topic, the scope and limits of science.

Hierarchy of sciences

Claims to understand anything about the early universe might seem presumptuous, but cosmology is actually one of the more tractable sciences. Inside a star (and in the early universe) conditions are so extreme that everything is broken down into its atomic constituents, and governed by simple laws. Moreover, the laws of physics are universal: atoms in remote galaxies and at early cosmic epochs obey the same laws as those we

study in the laboratory. It is complexity that makes things hard to understand, not size. Understanding a frog is a far more daunting intellectual challenge than anything confronting cosmologists.

The atoms that made the young earth are stardust – to have understood this is a triumph of twentieth century science. However, elucidating how those atoms combined, via Darwinian selection, into progressively more intricate forms, and eventually into creatures that could ponder their origin, is an unending quest that has barely begun. This perspective should caution us against scientific triumphalism – against exaggerating how completely we will ever understand anything really complex. The different sciences are in a hierarchy – in the complexity of the things they deal with – with physics at the base and (I suppose) psychology at the apex. But that does not mean that the other sciences are applications of physics. Every science, from chemistry to social psychology, has its own irreducible concepts, based on the emergent properties of complex systems. In understanding turbulence, analysing the fluid in atoms does not help. What goes on in a computer could be described in electrical terms, but that misses the essence, the logic encoded in those signals. We cannot solve Schrödinger's equation for any biological system, but even if we could, it would never yield an economical description or insight.

There is a sense – but a limited one – in which some sciences can claim to be especially 'fundamental'. Causal chains – if you go on asking why? why? – lead back to a question in particle physics or cosmology. These subjects relate to deep aspects of reality. We pursue them for the stimulus they pose (to theorists and instrumental innovators alike), and because the breadth has proved a fruitful policy – not because the rest of science depends on them. The sciences are not in a hierarchy whose superstructure is imperilled by an insecure base.

Status of scientific ideas

The way we approach science, what problems strike us as interesting, what styles of explanation are culturally appealing, and (more mundanely) what fields attract funding depend on a range

of political, sociological, and psychological factors. Some projects, especially big international ones, are a by-product of activities driven by other imperatives. (For example, the exploding supernovae I mentioned above would be less well understood were it not for the space programme, and supercomputers.)

It is important, as well as enlightening, to appreciate how pervasive these social and political factors are. The behaviour of scientists, and the external influences on their community, offer fascinating topics for study. However, for us 'in the zoo' science itself advances, albeit fitfully, towards a culture independent outcome. Steven Weinberg gives an apt metaphor:

A party of mountain climbers may argue over the best path to the peak, and these arguments may be conditioned by the history and social structure of the expedition, but in the end either they find a good path to the summit, or they do not, and when they get there they know it.

Why can we, seemingly, make some sense of the external world? Why can so much be described mathematically? Is mathematics itself a discovery, or an invention? These questions are for philosophers – they are deeper than scientists can professionally address. So let us turn to more concrete aspects of discovery.

Discoveries out of the path of imagination

Nearly 400 years ago Francis Bacon highlighted three astonishing discoveries – gunpowder, silk and the mariner's compass. In *Novum organum* he writes: 'These things... were not discovered by philosophy or the arts of reason, but by chance and occasion'; they are 'different in kind so that no preconceived notion could possibly have conducted to their discovery'. It was Bacon's belief that 'there are still many things of excellent use stored up in the lap of nature having nothing in them kindred or parallel to that which is already discovered... lying quite out of the path of imagination'.

So it was, most certainly, in the nineteenth century. X-rays, discovered just 100 years ago, must have seemed

fully as magical as the compass did to Bacon. Though of manifest benefit, they could not plausibly have been planned for. A proposal to make flesh appear transparent would not have got a research grant – even if it had, it surely would not have led to X-rays. It is easy to pick other examples. A nineteenth century project to reproduce music would have led to immensely elaborate pianolas or orchestrions, but would not have identified – still less accelerated – the technologies that actually achieved this goal.

Bacon contrasts his three 'magical' discoveries with the invention of printing, which 'has nothing in it which is not open and generally obvious... when it had been made, it seems incredible that it should have escaped notice so long'. Most innovations now emerge, as printing did, via Bacon's second route: 'from the transferring, composition and application of [things] already known'. What is remarkable, and indeed exhilarating, is that discoveries still take us unawares. There can still be scientific revolutions, despite the immense infrastructure of natural science that was quite lacking in Bacon's day, or even in the nineteenth century; indeed the lengthened frontiers of knowledge increase the chance of surprises.

I will focus below on some contemporary lessons that can be drawn from these distinctions, but will first make some general remarks on the creative impetus that drives any discovery or invention.

Creativity and individuality

Scientific insight needs concentrated effort and preparation. It also demands intuition and imagination. In this respect, it parallels artistic insight – equally an attempt to seek new patterns, and new perspectives on the world. However, those similarities should not obscure one glaring difference between the two enterprises, which stems from the interlocking, cumulative, and intensely social character of science.

In the arts, individuality shines through even at the amateur level. Everyone's contributions, even if soon forgotten, are personal and distinctive. Issues or priority do not arise, as they do in science. As Medawar noted, when Wagner took 10 years off in the middle

of the Ring cycle to compose the '*Meistersinger*' and '*Tristan*', he was not worried that someone would scoop him on '*Götterdämmerung*'.

In science, discoveries emerge when the time is ripe – they are contingent on some prior innovation, perhaps in another field. Individuals seldom make more than a few years' difference to when a particular advance occurs. There are exceptions – the laser for instance, or sociobiology – but in populous fields the key ideas are 'in the air', ready to be grasped. The greatest figures may, however (as Watson and Crick, for instance, did) help those ideas to gell neatly, rather than emerge untidily. What made Einstein unique in twentieth century physics is that he really did make a distinctive long lasting imprint: without him, we might have had to wait decades for equivalent insights on gravity.

Although their personal imprint may fade, scientists have the compensation that, if it survives critical scrutiny, their work endures as a brick in the edifice of 'public knowledge'. And they have a second compensation: literary critics are seldom also creative writers, but every researcher is, albeit in a small way, both a creator and a critic of the collective scientific enterprise.

These remarks about science, incidentally, are intended to embrace engineering and technology as well. Claims that 'academic' research demands mental processes of a specially elevated kind are baseless snobbery, of a kind that is thankfully dying out. There is at least as much innovation in the computer or pharmaceutical industry as in any field of basic research. Indeed, the intellectual leap in the concept of a zip fastener surpasses what most academics achieve in a lifetime.

Until the nineteenth century academic science (such as it then was) was only tenuously linked with practical innovation. Science and technology now have a complex symbiosis. Research triggers applications; but, equally, new techniques and instruments boost scientific discovery. If you look at what people do day by day, there may seem little difference between a biotechnology company and a university laboratory, not between someone in an aerospace company and someone innovating an instrument for space astronomy. These activities have something crucial in common: the work

is only worthwhile – it will only pay real 'dividends' – if it is really at the leading edge.

Forecasting and insight

In 1937 the American National Academy of Sciences organised a study aimed at predicting breakthroughs; its report makes salutary reading for technological forecasters today. It came up with some wise statements about agriculture, about synthetic gasoline, and synthetic rubber, but what is more remarkable is the things it missed. No nuclear energy, no antibiotics (though it was 8 years after Fleming), no jet aircraft, no rocketry nor any use of space, no computers; certainly no transistors. This committee overlooked the technologies that actually dominated the post-war era.

The pace of technological advance certainly is not slacking. Some everyday artefacts – laptop computers, camcorders, even supermarket barcodes – depend on basic science that dates back only 20 years or so. Likewise the new fields of biotechnology. A current attempt to predict future breakthroughs might have a 'hit rate' as dismal as the US forecasters achieved in 1937. The most dramatic and fruitful innovations will still surprise us. They will be the outcome of *some* new basic science, but of course we do not know what. Applications that are transforming the way we live were initiated by investments in basic research that were modest in relation to their impact. Some projects paid off colossally; others did not, but retrospective studies in the USA suggest that overall 'return' on basic research exceeds 20%.

On the football field, not everyone scores – you cannot predict who will, nor when. However, that does not mean that the other players are dispensable. Likewise, it is essential to maintain a broad science base, and a web of connections between different disciplines. Advances that deepen our understanding of some important aspects of nature – the physics of materials, living cells, or the environment – or even new mathematical ideas, are likely to find some application.

Obviously some fields are more likely to foster applications than others (I would concede that molecular biology is

a better bet than black holes in this regard!), but 'curiosity driven' research can impact in quite unexpected ways. For instance, it was studies of dust in interstellar space that led to the carbon structures known as fullerenes; and the exotic fauna on the ocean bed may seem as remote as outer space, but their ecology is relevant to the Brent Spar debate.

Nothing I have said, incidentally, is meant to disparage the Foresight exercise undertaken by the UK Government, whose agenda is less futuristic. The Foresight panels bring people together, forge new links that are themselves worthwhile, and will help the country to exploit what has already been discovered. A 'Foresight' strategy could do harm only if undue concentration on highlighted research areas led to a funding blight on others.

Climate for invention and discovery

The UK has a fertile record for 'leading edge' discovery and invention. Can we enhance it – can we at least ensure that we do not slide relative to others? and can we exploit our discoveries better?

'A society organised to allow and celebrate the creative spirit of science will find itself also productive of the other forms of creativity which make life worth living. The societies where the bursts of scientific energy occur...span the other arts too.' This quotation is from a lecture given last year by William Waldegrave¹; he went on to present a recipe, with which I strongly agree. We must reverse what he termed the 'Balkanisation of intellectual life – an affliction as acute in the humanities as in the sciences'. He recommended a broader education, interdisciplinary contacts in universities, and 'public understanding' programmes. However, current trends risk moving the other way – towards conditions *less* propitious for real innovative thinking, or for a more integrated culture.

The UK Government 1993 White Paper 'Realising our potential' rendered explicit the doctrine that science could be harnessed to wealth creation; and also to enhancing the quality of life. (Incidentally 'creating wealth' and 'enhancing the quality of life' are not really equivalent 'ends' that should be

equally extolled. The Newcastle philosopher Mary Midgley, who writes cogently on such issues, reminds us that much economic activity is valued as a means, direct or indirect, towards enhancing activities that we undertake for their own sake.)

Most of us would broadly assent to what the White Paper's principal authors meant. Indeed there are echoes right back to Bacon: 'the emolument of life... and the relief of man's estate'. Yet some things being argued in its name risk being unwittingly detrimental to these shared aims. Unduly *dirigiste* policies would quench real originality. Not only would this be bad for basic research in itself, but it could compromise the research universities, whose standards are crucial for the quality of expertise entering industry and the professions, as well as the flow of new ideas – precisely the activities that have (in retrospect) been their prime contribution to the economy. (My biomedical colleagues may be more sanguine because they have more options for supplementing governmental funds from other sources.)

Budgetary competition is obviously acute – between science and other public spending, and among the sciences themselves – but efforts to make research more accountable and impose a business based perception of efficiency could backfire, for three reasons. First, assessment exercises, grant reviews, and so on can become so time consuming that they seriously erode effort. Second, the most original lines of inquiry cannot always be 'packaged' into acceptable research proposals. Third (and more insidiously) the most creative and astute individuals may be discouraged from pursuing a research career altogether. It is a poor deal if top quality innovative output falls by a bigger percentage than the financial 'saving'. We need to be businesslike. So does a hospital – so does even a church – but that does not mean that we should operate too like a business.

Despite Britain's poor showing in other international league tables, we certainly do not lag Germany and Japan in the quality and range of our discoveries. Nor do we lag economically in industries like pharmaceuticals, where the research emphasis is strong. The challenge is to remedy our relative weakness

in other sectors without jeopardising what is already doing well. Scientists can be excused for thinking that the problem lies not primarily in themselves, but in the low research and development investment in some sectors, and companies that are not even receptive customers for research or enlightened employers of scientists.

Because the universities and research councils are public bodies, the government has been able to enhance the industrial influence on them. What is lacking is enough diffusion the other way. Perhaps we need to match Germany and Japan in the number of scientists and technologists on company boards.

An international opportunity

It is not only in Britain that mechanisms introduced to make research more efficient risk backfiring. A survey by the physicist Leon Lederman, when he was AAAS President, revealed how young US scientists perceived their prospects and pressures as being worse than their predecessors'.

Despite its alleged malaise, US science maintains great vitality. This is because its catchment area for talent extends worldwide. The USA is a magnet for the strongest graduate students and researchers. These come especially from Asia, but from Europe as well. (After the collapse of the Soviet Union, many leading Russian scientists moved directly to the USA.)

Why should we not try to match the blandishments of the USA for internationally mobile talent? All too often, one hears of people being 'lured abroad', as though this drain is something we must resignedly accept. Yet, this country has impressive advantages through the quality and tradition of our best research institutions, and we have an unmerited headstart over our European neighbours through the primacy of the English language.

UK universities have a fine record for attracting overseas students, but we should surely press it further. Why not aim to be the country of choice not only for undergraduates, but for top ranking graduates, and for the potential leaders of the international research community – those whose first instinct is now to go the USA? Everyone is aware of

the benefits of the right kind of inward investment. Britain could surely exploit more fully its manifest comparative advantage as a magnet for talent, a location for research centres, and an incubator for discovery and invention. The law of increasing returns surely applies: an extra 5% funding would yield much more than a 5% boost. Conspicuous success feeds on itself by stimulating and drawing in more expertise. As one of the 'societies where bursts of scientific energy occur' – to quote William Waldegrave's words again – we would gain all the correlated benefits that he identified.

Young people: the 16–18 curriculum

This leads to the worrying issue of school education, where our international rankings are low. My remarks above on 'public understanding' omitted one specially crucial segment of the 'public': those still in school. The British Association runs programmes, all through the year, to build on young people's natural enthusiasm for science and technology. It is keen to foster and cooperate with innovative schemes that bring individual research scientists in contact with schools. There is growing scope here: telecommunications allow remote access to large facilities, so that individuals – amateurs at home, as well as young people in schools – can participate in scientific discovery. Future scientists may be less corralled into large laboratories.

Sir Claus Moser's Presidential Address in 1990 led to the National Commission on Education, run under the association's auspices and funded by the Hamlyn Foundation. There have been moves towards implementing a few of the commission's recommendations – on nursery education, and on coordinating vocational qualification – but there has been little movement on most others. Sir Ron Dearing is now reviewing the system of qualifications at 16 and 18; we hope he will interpret his remit broadly, and note the commission's strong emphasis on broadening the A level curriculum.

Young people opting for humanities should not drop all science when they are 16. An appreciation of science is vital not just for tomorrow's scientists

and engineers, but for everyone who will live and work in a world underpinned by technology – and even more vulnerable to its failures and misapplications than the present one. Even more important, the option of higher education in science and technology should not be foreclosed to them. (These concerns pertain to England more than Scotland. Scottish education has its admirers here, but few in Scotland advocate a switch to the English system?)

It is crucial that enough of the brightest young people go on to acquire some professional expertise in science and technology. They will not do so unless, when making the key decisions at age 16 or 18, they perceive a range of appealing opportunities. They will be discouraged if the courses do not inspire them; or if the exciting discoveries reported in the media all seem made in the USA (or, worse still, by people from this country who have defected overseas). They will be discouraged if scientists seem valued less than accountants; and they need to feel that science is humanly relevant – that it meets their ethical concerns.

The OST and the DTI

A word now on the recent reorganisation of government science in Britain. The science, engineering, and technology community broadly welcomed the setting up of the Office of Science and Technology (OST) in the Cabinet Office. William Waldegrave, as its first minister, gained wide respect for his genuine commitment. We in the British Association especially appreciate the OST's continuing support for our mission of enhancing public understanding of science.

The shift into the Department of Trade and Industry (DTI) surprised most of us. It seemed to reverse one of the few government initiatives of recent years that commanded genuine bipartisan support. There had been no overt pressure for the move, certainly no open discussion. Some industrialists (Sir Richard Sykes among them) have argued that from their perspective, and that of smaller companies, the change could offer benefits. We hope the move indeed proves beneficial, but it would be remiss not to mention the worries

some association members have, in the hope that they will prove groundless.

There is concern that there should not be an undue focus on research with a short term payoff – this is not just because we value curiosity driven research for its own sake (though we do), but because past experience suggests that it is through free ranging basic research that the universities can make their most distinctive contribution to boosting industry and meeting other public demands.

There is also concern that its embedding within DTI should not hamper the OST's efforts at deploying the overall case for science, and coordinating policy across other departments. Strong links are indeed needed with industry, but it is also important to strengthen those with defence, health, environment, and (perhaps above all) with education. And the new Chief Scientific Adviser must have a real cross-departmental influence – Professor May, an old friend of the British Association, carries with him our good wishes and confident hopes. Also the Commons Select Committee should continue – it is an index of parliamentary interest in scientific and technical matters.

Science is not a monolithic profession; not a single constituency; certainly not a lobby. It is a pervasive activity. As with other long term issues (energy and environment, for instance), it is best if national science policy is bipartisan, rather than the subject of strident debate. However, the 'downside' is that science then loses visibility on the political agenda. The British Association, with its broad involvement, and its traditions as an informal 'parliament of science', is well placed to extend its role as a policy forum; I think it has a responsibility to do so. Let us hope that long term issues will generally loom larger as the millennium approaches.

The atomic scientists

I should like to devote a few final comments to broader (even global) issues of scientific concern. We have recently marked the anniversary of the atomic bomb – an invention as 'different in kind' from anything before as gunpowder seemed to Bacon.

Many physicists who had worked at Los Alamos during the war returned to

academic work straight afterwards. Some of them founded the *Bulletin of Atomic Scientists*, a journal with the aim of alerting the world to the dangers of an arms race, and the urgency of arms control. The cover page of each issue shows a clock, the closeness of whose hands to midnight indicates how precarious the world situation is (or is thought to be by the journal's Editorial Board).

At the time of the Cuba crisis, the clock was at only 3 or 4 minutes to midnight. It was there again in the mid 1980s. At that time, the main issues were how to reduce the ever present risk of escalation towards catastrophic nuclear war – by malfunction or miscalculation, even if not by premeditated strategy. The risk in a single year may have been small, but the probability would have mounted up if conditions had not changed.

The nuclear arms race, from the 1950s to the 1980s, was fuelled not only by misdirected resources, but by misdirected inventiveness. Virtually every innovation was quickly matched by the other side, and the arsenals ratcheted upwards. Yet throughout this time some scientists (the founders of the *Bulletin* being among them) were using their international influence to foster arms control. In particular, there was constructive dialogue at the 'Pugwash' conferences, named after the village in Nova Scotia where the first meeting was held. The physicists involved in such conferences were in numerical terms trivial compared with those in weapons laboratories, but they constituted an informal channel back to their governments, and were genuinely influential in instigating formal contacts – especially in the 1960s, before other East-West channels opened up.

Scientific input into policy: the transatlantic contrast

From the 1970s, scientists from a younger (post-war) generation became active in arms control debates; some of these went on to hold official posts, either in the USA or in Russia. However, such people have no real counterparts in the UK, where we still rely on independent voices from the Second World War generation, who have maintained their concern ever since.

The reasons for this transatlantic contrast are not hard to find. In the USA, there is a 'revolving door' between government jobs and universities. (or organisations like the Brookings Institute) whenever the administration changes. The American Physical Society, the Union of Concerned Scientists, and other organisations, can draw on this independent expertise to prepare (often influential) reports. There is also the Jason Group – physicists of the highest academic repute who meet regularly, for several weeks per year, bringing fresh minds to bear on issues relevant to the Defense Department. Jason Group reports are generally classified, but its members acquire a background and credibility that enhances their effectiveness in open discussion.

Over here, on the other hand, government science is generally a lifetime career. The more pervasive secrecy inhibits well informed open debate. Defence scientists form a rather closed world. My own experience illustrates this. I have had contact with many physicists from Los Alamos and Livermore (who attend academic conferences, and contribute to the open literature), but not with their British counterparts. Greater openness could actually strengthen the defence establishments themselves. Their primary activities will (one hopes) be throttled down, but they must be helped to retain wide competence; arms control and verification will demand innovative ideas in many fields (chemical and biological, as well as physics based areas like space technology).

These specific points about defence science are a symptom of something that is routinely deplored at British Association meetings – the meagre input of scientists into the general political process in this country. This precludes informed debate on technical issues where well thought out new ideas are needed – energy, environment, and transport policy, as well as defence. We could learn from US practice and set up 'Jason style' groups – leading scientists from academe or industry who would not merely sit on advisory committees, but commit themselves to carry out serious interdisciplinary studies. This is something the new Chief Scientific Adviser might consider; or else an independent foundation.

Another valuable innovation would be a forum or institution that could fulfil, in the scientific and technical arena, the role that Chatham House fulfils in foreign policy. This is something that the British Association itself, perhaps with partner organisations, could seek to implement.

Global risks

The clock on the *Bulletin's* cover has been put back to 17 minutes to midnight. In nuclear terms, the world seems on a longer fuse, but bewildering new risks now confront us. These may not threaten a sudden worldwide catastrophe – the doomsday clock is now a less good metaphor – but are, in aggregate, as worrying and challenging.

The nuclear arsenals must be safeguarded, and gradually dismantled. Nor must new weapons proliferate, of types that require only minor adaptations of legitimate technologies that every nation aspires to.

For most of the world's people, the ideological stances of East and West were always an irrelevant distraction from the immediate problems of poverty, and natural hazard. These 'threats without enemies' relate to global questions of environment, resources, and biodiversity: how can these be safeguarded without jeopardising the aspirations of the poorer nations? There is no case for a brake on technical advances – the need, rather, is to accelerate but redirect them.

The thrust of economic development must, urgently, shift towards a mode that is more equitable, environmentally benign, and sustainable. This was a theme of Dr Anne McLaren's Presidential Lecture last year. She reminded us that education (especially for women) is a priority worldwide, not only for its own sake but because of its impact on family size. Stemming population growth may be a prerequisite for attaining acceptable quality of life everywhere.

I should like to quote related sentiments from a different quarter, the Prince of Wales. In a lecture at Cambridge University, he said:

The strategic threats posed by global environment and development problems are

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the most complex, interwoven, and potentially devastating of all the challenges to our security... Scientists... do not fully understand the consequences of our many faceted assault on the interwoven fabric of atmosphere, water, land, and life in all its biological diversity. Things could turn out to be worse than the current scientific best guess. In military affairs, policy has long been based on the dictum that we should be prepared for the worst case. Why should it be so different when the security is that of the planet and our long term future?

When the risks are global, the obligation to inform and to seek solutions extends to the international scientific community. Current concerns are much more diverse than those of the Cold War era, and cannot be tackled without wider expertise. Scientists in this country might have been reluctant to dedi-

cate their energies to *nuclear* arms control issues – in the ‘superpower’ confrontation, nothing Britain said seemed to count for much – but now that the agenda has changed, there need be no such inhibition. Britain has impressive credentials and a high international profile in the broad range of expertise that these global problems entail – problems that should loom as large in the political agenda as did the East-West political divide in the Cold War era.

Conclusion

I shall close with another brief cosmic perspective. Planets like ours – ‘cycling on’, in Darwin’s words, ‘according to the fixed law of gravity’ – are surely very common in the universe. But those

that harbour such *complexity* as ours could be surpassingly rare. The intricate biosphere, of which we are part, has taken several billion years to evolve, but in terms of cosmic timespans, we are not yet at the halfway stage – we could still be nearer to Darwin’s ‘simple beginning’ than to the endpoint of the evolutionary process.

It is, primarily, collective human actions that will determine how, or even if, that process unfolds. Being mindful of these potentialities stretches our horizons: it should deepen our commitment to understand our world, and conserve its web of life.

Literature cited

1. Waldegrave, W., ‘To communicate across disciplines’, *Interdisc. Sci. Rev.*, 1994, 19, 117–120.

CONTACT PROGRAM ON INTERFACES OF CHEMISTRY AND BIOLOGY for M Sc students

Date: 28 October to 8 November, 1996

Place: Mumbai

This program aims to provide an opportunity to final year students of M Sc (Chemistry, Biochemistry, Biotechnology and related areas) or B Tech (Chemical Engineering and related areas) to get to know some of the frontier topics of research interest at the interface of Chemistry and Biology and to interact with some leading scientists in an informal atmosphere. The program will attempt to give a flavour of topics such as Structure of biomolecules, Biophysical methods, Enzyme mechanisms, Enzyme inhibitors and drug design, Enzyme models, Lipids and biological membranes, Nucleic acids and molecular biology, and Molecular electronics. Lectures on relevant topics will be given by leading researchers and the students will be encouraged to take part in the scientific discussions. The participants will visit various well-equipped laboratories to introduce them to some of the latest techniques. They will also be involved in experimentation, familiarizing and learning advanced computational and instrumental methods of analysis.

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Essentials of Nuclear Chemistry. H. J. Arnikar, Fourth edition. Wiley Eastern Limited, 4835/24, Ansari Road, Daryaganj, New Delhi 110 002. 498 pp. Price: Rs 155.

The book presents a wide spectrum of topics, and some kind of comprehensive coverage on some of these, in the broad areas of nuclear-, radiation-, and radio-chemistry. It attempts to reach far beyond the 'essentials' of nuclear chemistry. The text is so much marred with typographical errors, conceptually wrong statements, grammatical errors and mis-information, that a student would be better off if he is not lured into reading this book, attracted by its charming appearance and get-up.

The author very often shows a lack of concern about the reliability and correctness of the data that are drawn from various sources. On page 7 (footnote) the half-life of proton decay is quoted from a 1981 unpublished work. This was OK for the first edition of the book published in 1982. But in 1995 the picture is very different. On page 403 (footnote) the ^{14}C specific activity in modern wood is quoted to be 13.6 dpm/g and to be controversial from a reference that is given in a text book of nuclear physics published in 1979. This issue must have been resolved by now. Taking another example, the oldest terrestrial rocks are stated to be 2.7×10^9 y, and the age of the earth is given as $(5.0 \pm 0.50) \times 10^9$ y, (p. 407), both values from a 1954 text. Even in 1981, these values had become obsolete. Much progress has been made in this field.

Examples of conceptually erroneous presentations:

(i) Page 4, line 3 from bottom: 'An atomic nucleus, considered without relationship to the outer sphere, is often referred to as a *nuclide*....'

(ii) Page 5, Section 1.3.1 (b) '*Isobars*: These are nuclei of different neighbouring chemical elements having....'

(iii) Page 8, line 11: 'This suggests.... the charge independent nature of the nucleons in respect of stability'.

(iv) Page 23: In Figure 2.1 the ordinate should be ρ/ρ_0 . (not ρ , as given) and the number 4.40 should be replaced by 4.4α .

(v) Page 23, Figure 2.2: The ordinate label 'Nucleon Density (Relative)' should be changed to 'Nucleon Density; 10^{44} nucleons/ m^3 '. The ordinate scale should have values from 0 to 2.00.

(vi) Page 25, eq 2.7b. It is incorrect to say that in this equation a , and b are constants.

(vii) Page 40, Section 2.92: 'If all the energy levels in an assembly of molecules.... were equally populated, no resultant magnetization would occur on the application of an external field.'

(viii) Page 41, line 3: 'In 1.8 g of water the numbers (of molecules) in the lower and higher energy states will be of the orders of 10^{23} and 10^{18} respectively'.

(ix) Page 54, para (b), line 4: 'Generally, a single crystal of sodium nitroprusside.... is adopted as a standard...'

(x) Page 56, para (c), line 2: 'However, when the environment is asymmetrical a feeble internal electric field operates and this leads to a quadrupole splitting of the line....'

(xi) Page 58, para (g) line 3: '...(The Curie point)... is also the temperature below which the single Mossbauer line splits into six lines due to a sharp decrease in the electron density at the nucleus.'

(xii) Page 83, Figure 3.3(b): The meaning and usefulness of $V_c(r)$ as shown in the figure, is not found anywhere in the text.

(xiii) Page 134, last line: An incorrect expression, $e^{\lambda_1 - \lambda_2}$, has been used. The exponent must be dimensionless. This mistake also occurs in the first edition of the same book by the author.

(xiv) Page 243, line 4 from bottom: '... the yield of symmetrical fission... is the lowest...'. This is not so.

(xv) Page 246, para 6.5 line 4: '... fission fragments.... fly apart with velocities much in excess of those of their orbital electrons.'

(xvi) Page 294, second sentence: 'Excepting γ which is purely an electromagnetic radiation, and neutrons which are uncharged, all other forms of radioactive samples emit charged particles as β^\pm , p, d, t, α ,... whose detection ...'.

(xvii) Page 182, Problem 4.1: '...1 g of ^{226}Ra emits ... atoms of radon' Using similar words can we say that ' ^{14}C decays by emission of nitrogen?'

(xviii) page 364 Section (b): '.... ^{14}C are formed continually in nature by an alternative interaction of fast neutrons of cosmic origin with the atmospheric nitrogen.'

(xix) Page 383, Equation 10.22: Using this equation and the data that are given, b comes out to be 0.88×10^{-16} and not 4.11×10^{-16} .

(xx) Page 430, Equation 11.3: The equation $\text{LET} \propto Mz^2/E$ is interpreted by the author as 'The above expression shows that LET increases upto a maximum as v decreases along the path, after which the LET decreases along with v .'

(xxi) pp 405–407, Section (c); Age of Minerals and Rocks: The matter presented in this section is erroneous on almost every point. The relation.

$$M = N_1(e^{\lambda_1 t} - 1) + N_2(e^{\lambda_2 t} - 1) \dots 10.38$$

has been simplified by the author to: $M = (N_1\lambda_1 + N_2\lambda_2)t$, and is used, on pages 406 and 407, to calculate the age of granites and the age of earth's crust using ^{40}K as a chronometer, whose half-life is 1.28 Gy. Since, for the values of ages involved, the exponent in eq. 10.38 is greater than 1, the adopted simplification is invalid. The author fails to note this, and using the invalid but simple expression, calculates the ages of granites, and that of the earth's crust respectively, as 10.1 and 8.6 Gy. He tries to cover the discrepancy by 'suggesting an overestimation of radiogenic calcium content'. He 'explains' the high K–Ar age by stating that 'the bulk of the present day Ar in the atmosphere was released thereto while the surface of the earth was still in the fluid form'. Such a scenario would, however, give a lower radiogenic argon and would go in opposite direction. The author, irrelevently, brings in the name of Professor Urey here – blatant misinformation.

(xxii) Pages 232–236, Section 5.12; The origin and evolution of elements.

This section is entirely incorrect and full of misconception. Ideas that have been found to be untenable are presented as valid explanations, while some of the well-accepted processes are presented with such distortions that they lose their meaning. We know that nucleosynthesis of elements beyond helium is not possible in Big Bang. The suggestion, presented in Section 5.12.3, that the medium weight elements were

BOOK REVIEWS

formed by fission of heavy elements fails to account for the presence of the p-process nuclides and has never been taken seriously.

Examples of some sentences that suffer from 'language problem':

(i) Page 79, para (f): 'The energy discontinuity is around 2 MeV, i.e. about 25% of mean binding energy in β disintegrations in the neighbourhood of magic numbers.'

(ii) Page 269, second para, last sentence: 'Since then ever so many reactors have been functioning in many countries at very many times higher power levels, extending upto 10^9 W.'

(iii) Page 227, first para, second sentence: 'This is in spite of its advantages in respect of the raw materials needed being very much cheaper and the process involving far less of radiation hazard as none of the by-products is radioactive which is not wholly contained.'

(iv) Page 290, third para: 'Let us note that reactor or no reactor, mankind has been exposed from the beginning of time to nuclear radiations of cosmic and terrestrial origin, besides those due to ^{40}K present in all rocks and the seas and in our very body, besides ^{14}C .'

P. S. GOEL

117/O/337, Geetanagar,
Kanpur 208 025, India

Wheat Revolution – A Dialogue. M. S. Swaminathan (ed.). Macmillan India Ltd., P.B. No. 7092, Daryaganj, New Delhi 110 002. 1993. 164 pp. Price: Rs 224.

This book contains the history of growth of wheat production in India, leading the country not only to self-sufficiency but to food security. Revolutions are a radical change in the constitution of a country after revolt, which are often violent, and after a change in government dissipate over time. 'Wheat revolution', however, saved the country from going through a revolution as was predicted by some writers from developed countries. Has the 'Wheat revolution' dissipated as pointed out by Mr.

Shivaraman? Therefore, recapitulations of events, in the form of a dialogue by major actors which led to 'Wheat revolution' synonymous with green revolution was an appropriate and laudable effort. M. S. Swaminathan, who organized the dialogue at M. S. Swaminathan Foundation, and had the central role in it, is to be commended for bringing out this book for the benefit of present and future generations.

Several questions have been raised in the recent past about 'Wheat revolution/Green revolution' relating to leadership, scientific basis and policy actions. The book provides answers, to some extent, to these questions through group discussion on (a) Package of technology, including new varieties, agronomic practices and post harvest handling, (b) Package of services, including the timely supply of seeds, fertilizer, water and credit and (c) Package of public policies, assured and remunerative marketing, building of grain reserves, etc.

It is obvious from the discussions that till 1963 there was no technology when the seeds of varieties having Norin dwarf traits in spring wheat background were obtained in sufficient quality from Borlaug to plant experiments at IARI (Indian Agricultural Research Institute, New Delhi). Since this material was found promising at Delhi, Ludhiana and Pantnagar, a decision was taken to put demonstrations in farmers fields, thus by-passing the usual norms of coordinated trials and extension programmes. This speaks of the confidence of scientists in adopting an alternate strategy. A large amount of credit goes to decision makers, particularly C. Subramaniam who agreed for the import of 18000 tonnes of wheat seed from Mexico of the varieties which were already identified at Delhi, Ludhiana and Pantnagar. This material was planted in Punjab (then Punjab and Haryana) and Western UP where the climate was suitable for wheat production and supported by assured water supply, and enterprising farmers. Within three years production jumped from 12 to 17 million tonnes from 1965 to 1968. There would have not been so much success if the decision to purchase red wheat by government at the same price as desi wheat was not taken because the red grain of new varieties did not fetch good price in

open market. Even today the amber varieties derived from Mexican dwarfs such as Kalyansona, HD2329, HD2285 and others fetch less price than desi varieties such as C306, NP824, K68 and others. Thus the purchase price was an important decision for encouraging wheat production. Having got success with production, it was necessary to develop varieties with amber grain colour as against the red colour and chapati quality acceptable to consumer. Interestingly, a population S227 which came from Mexico provided several amber grain selections such as S227, S307, and S308. The selection S227 was named as Kalyansona which had performed very well at Delhi, Ludhiana and Pantnagar. Kalyansona was an acceptable variety for grain colour but still required improvement for chapati quality. Later on several crosses using Indian varieties and new dwarf varieties were made and varieties with better quality were developed. The technology with reference to irrigation and fertilizer application was developed. It so happened that 1964 and 1965 had deficient monsoon rains, and possibly a kharif crop preceded wheat crop, therefore, six irrigations appeared to produce the maximum yield. At that time not much attention was paid to soil-moisture profile and winter rains. However, the recommendation of five to six irrigations was extensively popularized and made sacrosanct. This led to over-irrigation and with considerable difficulty, now, the reduced number of irrigations are an acceptable recommendation. It also took time to fix an economic dose of fertilizers which is dependent on location, variety, irrigation, etc. The most fortunate aspect of wheat production in India is that we have no insect pest problem and the Indian scientists from the time of K. C. Mehta and B. P. Pal have been experts in breeding for rust resistance.

The 'package of services' includes the recommended seed for a given location, irrigation, fertilizer availability, and any other input such as machinery or energy which now have become important. In the initial phase 1966 to 1968, the target area was Punjab (Punjab and Haryana) and West UP where most of these services were available. However, there was a coordinated effort among scientists, bureaucrats, politicians and above all

the farmers. The need of the hour was to increase production and hence everyone made his or her contribution to this national goal. The 'dialogue' brings out clearly that the scientific awareness, vision and intuitions of M. S. Swaminathan, combined with clarity and commitment to national objectives of C. Subramaniam were the major contributors to 'wheat revolution'. The late B. P. Pal gave all the encouragement to scientists and the late V. S. Mathur bred a number of varieties which even today

are the dominant varieties. The country owes them gratitude for their accomplishments. There was, however, a significant role of the Indian Agricultural Research Institute, New Delhi, Punjab Agricultural University, Ludhiana and G. B. Pant University of Agricultural Sciences & Technology, and their scientists who worked together to make a success of dwarf wheats in India. Norman Borlaug through his material and visits to encourage scientists played a pivotal role in this programme.

There are some activists in the country who have been critical for wheat/green revolution for various reasons. They are entitled to do so because they have enough food available to them and have rarely experienced the scarcity of food.

S. K. SINHA

*Water Technology Centre,
Indian Agricultural Research Institute,
New Delhi 110 012, India*

COSTED-JNCASR Fellowship Programme

This Fellowship Programme has been jointly instituted by the Committee on Science and Technology in Developing Countries (COSTED) and the Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR) to foster free mobility and exchange of scientists in developing countries and to promote South-South co-operation. The JNCASR, operating under the direction of Prof. C. N. R. Rao, FRS, is a centre of excellence in Bangalore, India and essentially works through close links with several reputed academic scientific institutions and centres in India.

The fellowship covers short-term research, training or participatory research work in physical, chemical and biological sciences in reputed scientific institutions in India including the JNCASR. The programme is open to scientists, teachers and research scholars in the developing countries in the Asian, African, Arab and Latin American regions (except India).

The duration of the fellowship is up to 3 months. The fellowship covers free lodging as well as an adequate allowance in Indian currency to cover boarding. A few travel grants are available for exceptionally meritorious candidates.

The applicant must be a scientist, a teacher or a research scholar affiliated to a scientific or academic institution and below 45 years of age. For further information on the programme, contact:

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MEETINGS/SYMPOSIA/SEMINARS

Contact Programme in Ecology

Date: Second half of August 1996
Place: Varanasi

The programme aims to expose M Sc students of Botany/ Environmental Sciences/Life Sciences and related subjects to different techniques involved in ecological studies with special reference to Environmental Impact Analysis.

Contact: Dr Madhoolika Agrawal
Convenor
Department of Botany
Banaras Hindu University
Varanasi 221 005
Phone: 310293 Ext. 352
Telex: 545304 BHU IN
Fax: 91-0542-312059

Third SERC School on Seismology and Earthquake Processes

Date: 23 September – 12 October 1996
Place: Dhanbad

Theme: Computational seismology and induced seismicity. The course content includes: Fundamentals of seismic wave propagation, Seismometry and accelerometry, Observational seismology, Focal mechanism of earthquakes, Seismicity and seismic zoning, Fractal analysis of earthquakes, Digital seismograph and telemetry, Signal processing, Data analysis—digital and statistical, Reservoir-induced seismicity, Mining-induced seismicity, Special lectures on topical themes.

Contact: Prof. R. K. S. Chouhan
Course Director, SEP III
Indian School of Mines
Dhanbad 826 004
Phone: 0326-202577, 202578 Ext. 220
Fax: 0326-203042, 202380

SERC (DST) sponsored Fourth Winter School on databases, Numerical Methods and Computer Modelling in Modern Approach to Petrology

Date: 19 November – 9 December 1996
Place: Dehra Dun

Contact: Prof. K. K. Sharma
Course Director, NMCMP-IV
Wadia Institute of Himalayan Geology
General Mahadeo Singh Road
Dehra Dun 248 001
Telex: 0585-326 WIHG-IN
Fax: 091-0135-625212
E-mail: wihg@sirnetd.ernet.in

National Conference on Pollution Control and Management in Coal Mining and Thermal Power Plants

Date: 22–24 December 1996
Place: Jyotivihar

Themes include: Occurrence of trace elements in coals; fate of trace elements during coal combustion; Environmental impact of coal mining and thermal power plant; Pollution control and management in coal mining areas; Pollution control and management in thermal power plants.

Contact: Director
National Conference
Department of Environmental Sciences
Sambalpur University
Jyoti Vihar 768 019
Phone: 0663-430301

Silver Jubilee Meetings of the Ethological Society of India and National Symposium on Behaviour

Date: 28–30 December 1996
Place: Raipur

Topics include: Nutritive behaviour; Rhythmic behaviour; Communicative behaviour; Social behaviour; Reproductive and breeding behaviour; Adaptive behavioural physiology; Environment and behaviour; Hormones and behaviour; Foraging behaviour; General and applied behaviour.

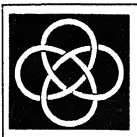
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Organizing Secretary
Silver Jubilee Meetings of ESI
and National Symposium on Behaviour
School of Life Sciences
Pandit Ravishankar Shukla University
Raipur 492 010
Phone: 91-771-26031
Fax: 91-771-534283; 91-771-534990

84th Indian Science Congress (Indian Science Congress Association)

Date: 3–8 January 1997
Place: Delhi

Focal theme: Frontiers in Science & Engineering and their Relevance to National Development.

Contact: Dr S. Das Gupta
Executive Secretary
Indian Science Congress Association
14, Dr Biresh Guha Street
Calcutta 700 017
Phone: 240-2551, 247-4530
Fax: 0091-33-2402551,
Telex: 021-5224 ISCA IN



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7. Names and addresses of two referees
8. Educational qualifications with complete details:

Name of Institution/Board	Year of passing	Exam/Degree	Main subjects	Marks % in aggregate and division
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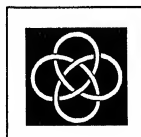
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Name and Address of Employer/Instt.	Period of service From-To No. of yrs. and months	Post held, pay and scale of pay	Whether permanent or temporary	Reasons for leaving
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10. Any other relevant information.

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The Senior Administrative Officer,
IUCAA, Post Bag 4, Ganeshkhind, Pune 411 007.



INTER-UNIVERSITY CENTRE FOR ASTRONOMY AND ASTROPHYSICS

An Autonomous Institution of the University Grants Commission, India.

The Inter-University Centre for Astronomy and Astrophysics is setting up an optical near-infrared observational facility with a 1.5-2 m size telescope. In order to carry out development of various instruments for the observations, the Instrumentation Laboratory of the Centre is looking for a YOUNG PHYSICIST, for the position of Scientist C, with a Ph D in any branch of experimental physics and having an aptitude for instrumentation. The selected candidate would be placed in the scale 3000-100-3500-125-4500, with the usual allowances applicable to central government employees stationed at Pune.

Further, as the Giant Metrewave Radio Telescope of the National Centre for Radio Astrophysics is nearing completion, IUCAA is also looking for a YOUNG RADIO ASTRONOMER in the same grade with a Ph D in radio astronomy, to help initiate any joint projects with the NCRA.

Please apply to the Director, IUCAA, P. B. No. 4, Ganeshkhind, Pune 411 007, with biodata and a list of at least three referees. The applicants should ask these referees to send their confidential recommendations directly to the Director.

Using Agricultural Diversity Research Award

The Award

The Using Agricultural Diversity Award assists groups and institutions in India, Nepal and Bangladesh to undertake applied research on the use of agricultural diversity to meet farmer needs. The award is intended to encourage research collaboration, exchanges and dissemination of information on practical means to widen the range of crop varietal choices available to farmers.

Eligible Fields of Study

Applications will be accepted for applied research on topics related to:

- Testing and developing participatory approaches and methods for enhancing the on-farm use of agricultural diversity.
- Collaboration among groups currently engaged in on-farm conservation and enhancement of agricultural diversity.
- Informing policy of the role of farmer participation in on-farm conservation and enhancement of agricultural diversity.

Program Areas

Awards will be given in three Program Areas. Applicants must indicate the program area for which they wish to be considered, and may not apply to more than two program areas in the same cycle of competition.

Research Program

The Award will be used to support activities that seek to:

- Improve the practice and extend awareness of participatory crop selection and breeding efforts/or,
- Enhance the dynamic nature of community level seedbanking and seed supply initiatives by increasing the range of crop varieties available to farmers so as to meet changing needs/or,
- Explore links between community seedbanks and national or regional genebanks in order to improve seed security/or.

Exchange Program

The Award will be used to facilitate systematic exchanges among groups in South Asia engaged in or concerned with the conservation and enhancement of agricultural diversity on farm. This could include field visits by individuals to project sites, joint diagnostic and monitoring exercises involving more than one institution, consultation with specialists regarding methodological issues or the design and development of collaborative research projects.

Documentation and Dissemination Program

The Award will be used to undertake activities which contribute to the documentation of particular experiences with using agricultural diversity on farm and for disseminating the results of institutional activities, with a special emphasis on informing policy of participatory approaches and methods involving the conservation and enhancement of on-farm

agricultural diversity. Preference will be given to dissemination activities that make use of the internet and other forms of electronic mass-communication.

Eligibility

Applicants must meet the following conditions for eligibility:

- Research is in one of the eligible fields of study;
- Provide evidence of affiliation with an institution or organization in the region in which the applied research will take place;
- Provide evidence of previous work in the field of agricultural diversity conservation and use.

The award is principally to cover applied research costs; it cannot be used for permanent salaries or for the purchase of vehicles and other major fixed assets.

Duration of Tenure

Award tenure corresponds to the period of proposed activities. In general, this will be not more than 24 months.

Value

The Award will cover justifiable applied research expenses to a maximum of Rs 350,000.

Deadlines

There are two cycles of competition. All supporting documentation must be complete before the application will be considered. The deadlines for receipt of applications for each cycle are:

October 1, 1996 (awards will be announced mid-January)

February 1, 1997 (awards will be announced mid-April)

Applications

The Award is governed by a Steering Committee comprised of individuals from a wide range of national, regional and grassroots organizations in India, Nepal and Bangladesh as well as representatives from donor agencies.

Applications should be sent to:

Prof. S. K. Sinha

Chair, Steering Committee

c/o International Development Research Centre (IDRC)

17 Jor Bagh

New Delhi, 110 003, India

Fax: 91-11-4622707

email: UDRA@idrc.ca

This award is supported financially by the International Research Development Centre (Canada) and the Overseas Development Agency (United Kingdom)

Application Form
Using Agricultural Diversity Research Award

Name and Address:

Please submit all documents listed below. All documents must be received before applications are considered.

1. Summary of Research proposal (approximately 10 pages, not inclusive of bibliography) including:
 - project title and program area for which you wish to be considered
 - brief description of agricultural biodiversity in the region where the research is to be undertaken
 - explanation of how farmers perceive the use of agricultural biodiversity in their system of agriculture
 - research objectives
 - methodology
 - tentative schedule of field activities
 - ethical considerations
 - expected results and proposed monitoring mechanisms
 - potential use of results
 - possible beneficiaries of the research
2. Budget for proposed research
3. Application form (this page)
4. Curriculum vitae of principal investigator and co-investigator (if any)
5. Two references who will act as advisors (with full addresses)
6. Letter from institution confirming affiliation
7. Proof of citizenship or permanent residency

INFORMATION FOR CONTRIBUTORS

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All manuscripts should be addressed to the Editor, *Current Science*, P. B. No. 8001, C. V. Raman Avenue, Bangalore 560 080. Submission of an article will be held to imply that it has not been previously published and is not under consideration for publication elsewhere; and further, that if accepted, it will not be published elsewhere. *Three copies of contributions of all categories* are required, with a letter of transmittal giving (i) names and complete addresses of the authors and (ii) title of the contribution and the category in which it is submitted (see below).

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Review articles (not exceeding 5000 words) are expected to survey and discuss recent developments in a field. They should be well focused and organized, and avoid a general, 'textbook' style.

Research articles (not exceeding 4000 words) should report research results of fairly major significance. They should include an abstract not exceeding 100 words, introductory paragraph(s), and brief subheads.

Research communications (not exceeding 2000 words) should contain important findings that are novel and of fairly broad interest. They should include a brief abstract and an introductory paragraph. Text should not be broken up under subheads.

Correspondence includes letters, not exceeding 500 words, that are of general interest to scientists. All letters cannot be published.

Scientific correspondence contains technical comments, including those on articles or communications published in *Current Science* within the previous six months. Letters may be reviewed and edited.

Research news articles are intended to inform nonspecialists about recently published advances or important findings discussed at a meeting. Authors should also send a copy of the paper(s) on which the article is based. Meeting reports should avoid merely listing brief accounts of topics discussed, and must convey to readers the significance of an important advance.

Research accounts articles are intended to be personalized reviews of research from the authors' own laboratory, based on a body of published work. The articles must provide appropriate background to the area in a concise introduction, which should also serve to place the author's work in proper perspective. Articles will normally

not exceed 8 to 10 printed pages.

Opinion articles present views on issues related to science and scientific activity. **Commentary** articles should contain expository notes on issues related to science and scientific activity.

Book reviews. Unsolicited reviews will also be considered. Reviews that merely 'list' brief descriptions of the contents cannot be published. Reviews should have 'context' and convey some information about the subject of the book.

Historical commentary and notes inform readers about interesting aspects of personalities or institutions of science or about watershed events in the history/development of science; most are expected to relate to India. Illustrations are welcome. Brief items will also be considered.

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Manuscripts should be typed double-spaced on one side of white bond paper (21×28 cm). The pages should be numbered consecutively, starting with the title page and through the text, reference list, tables and figure legends. The title should be brief, specific and amenable to indexing. Not more than five **keywords** should be indicated separately; these should be chosen carefully and must not be phrases of several words. **Summary** and **abstract** should not have more than 100 words and should convey the main point of the paper, outline the results and conclusions, and explain the significance of the results.

Text. All papers should have a brief introduction. The text should be intelligible to readers in different disciplines and technical terms should be defined. Tables and figures should be referred to in numerical order. All **symbols** and **abbreviations** must be defined, and used only when absolutely necessary. Superscripts and subscripts and ambiguous characters should be clearly indicated. **Units of measure** should be metric or, preferably, SI. Methods should, as far as possible, be described briefly in appropriate table and figure legends.

Figures. In the case of line drawings one set of originals (without any lettering) is sufficient, with two copies containing lettering. In the case of photographs good prints are required with each copy of the manuscript; photocopies are not acceptable. Line drawings should be roughly twice the final printed size. The correct orientation should be indicated if not clear.

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References should be numbered in the order in which they appear, first through the text and then through table and figure legends. The following are examples of ways of writing references.

1. Mukundan, T. and Kishore, K., *Curr. Sci.*, 1991, **60**, 355-362.
2. Constantine, G., in *Biology of Bats* (ed. Wimsatt, W. A.), Academic Press, New York, 1970, vol. 1, pp. 319-322.

Acknowledgements should be brief. Footnotes are not allowed except to identify the corresponding author if not the first.

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1. Mukundan, T. and Kishore, K., *Curr. Sci.*, 1991, **60**, 355–362.
2. Constantine, G., in *Biology of Bats* (ed. Wimsatt, W. A), Academic Press, New York, 1970, vol 1, pp. 319–322.

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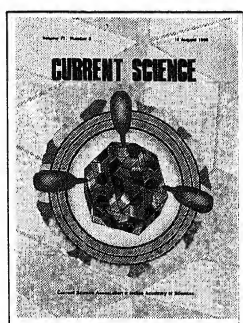
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COVER. Coated vesicle encapsulating a virus. See page 193.

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In this issue

How heavily does it rain in India?

Which place in India holds the record for the heaviest rainfall – Cherrapunji, Bombay or Mawsynram? Almost all of us will mark Cherrapunji as the correct answer. However, as described by P. R. Rakhecha and P. R. Pisharoty in their article on **page 179** of this issue, all the three places can legitimately vie for the first position.

One can talk of rainfall at various temporal scales – an hour, a few days, or the whole season; and interestingly, three different places qualify for the top slot in these three categories. Using the rich collection of the data at the India Meteorological Department, Rakhecha and Pisharoty have brought out some fascinating aspects of the heavy rains seen in the Indian monsoon season. Particularly intriguing are the depth–area–duration values associated with cyclonic storms. Even one look at a typical entry is enough to conjure up visions of a 100 km × 100 km area, completely covered to a depth of over half a meter of water in a matter of a couple of days.

Thunderstorms, responsible for heavy rains lasting for an hour or two, on the other hand, focus their attention on a much smaller area. Furthermore, thunderstorms of different intensities seem rather arbitrarily distributed across India. Who would have imagined the rainiest hour recorded in Hyderabad to be wetter than the one recorded in Kodaikanal or in Trivandrum or in Mahabaleswar? See **page 179** for more such surprises.

N. V. Joshi

Fighting the common cold

To sufferers of the common cold, the inability of the pharmaceutical industry to develop either a vaccine or an effi-

cient cure has always been a source of puzzlement. Grandmother's remedies and benign neglect are frequently the best course of action, although Pauling's followers still insist that vitamin C works wonders. Has modern biology contributed to understanding this widespread and most annoying affliction? On **page 193**, Michael Rossmann reviews the spectacular success of crystallography in delineating the structure and receptor interaction of a human rhinovirus, which belongs to a family of causative agents that are responsible for common colds. As far as biological organization is concerned, viruses are amongst the simplest organisms; however their atomic level structures are easily the most complex to have been determined by crystallography so far.

The rhinovirus structure reveals a 'deep crevice or canyon' on its surface which is the site for recognition by a cell surface viral receptor; permitting entry of the sub-viral particle or viral genome into the host cell. Here, the viral genes can be replicated, eventually leading to viral multiplication, resulting in a new generation of infectious particles. Rossmann's story is rich and detailed, providing insights into virus–receptor interactions. For the rhinovirus, the cellular receptor, which permits the infectious agent to home in, is the intercellular adhesion molecule-1 (ICAM-1), a complex membrane bound protein belonging to the immunoglobulin superfamily. The 'canyon hypothesis' for the receptor binding site on the virus immediately suggests possibilities for blocking the pocket and hence promises rational development of new anti-viral agents. Unfortunately, drug-resistant mutations can quickly develop, where the virus modifies the residues on the canyon walls and floor to preclude binding of potential therapeutics. The importance of the 'canyon' on the rhinovirus for receptor binding also rationalizes the inability of viral antibodies to efficiently inhibit virus–receptor interactions. The virus is cleverly able to hide the crucial bind-

ing site from immune surveillance. The battle against pathogenic organisms is clearly unending, with the enemy displaying formidable capacities for deviousness. In the case of the common cold, the advantage undoubtedly still rests with the rhinovirus.

P. Balaram

Microbe–drug wars

Drug resistance is a growing public health threat, with pathogenic organisms quickly learning to cope with the challenges posed by therapeutic agents. The strategies used by microorganisms include enzymatic degradation of the drug molecule, as in the case of penicillin resistance, where β -lactamases break down the antibiotic. Alternatively, clever microbes even alter the target structure as exemplified by vancomycin-resistant bacteria, which have modified cells walls containing lactic acid instead of alanine. This simple device eliminates a stabilizing hydrogen bond to the antibiotic, preventing its binding to cell wall precursors. A third stratagem involves membrane bound proteins which function as pumps and extrude the drug from the target cell. The problem of multidrug resistance in bacterial infections and cancer chemotherapy stems from the ability of the target cells to use the membrane bound pump as a 'hydrophobic vacuum cleaner' to sweep largely apolar organic drugs out of the cell. The understanding of multidrug resistance genes and their protein products has been growing, as reviewed by Rajendra Prasad *et al.* on **page 205**. Indiscriminate use of antibiotics and other therapeutics, compounds the drug resistance problem. The importance of developing new generation therapeutics cannot be over emphasized.

P. Balaram



**Indian Institute of Science
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Applications are invited from Indian nationals preferably below the age of 35 years, for faculty positions at the level of Assistant Professor in the Department of Electrical Engineering. The candidates, if selected, are expected to contribute effectively in the teaching and research programmes of Department which are generally in the areas of Power Systems, Power Electronics, Systems Science and Signal Processing.

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CURRENT SCIENCE

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CORRESPONDENCE

Merit and mockery

Most academic and scientific jobs in our country are available in government organizations. Over the years, the ratio of the number of job seekers to the number of available vacant positions in these sectors has increased many fold. However, given the importance of these sectors for developing human resources and strengthening scientific and industrial bases, meticulous recruitment of meritorious, innovative and efficient persons is essential. Unfortunately, in most cases, these criteria are overlooked.

Whenever vacancies arise for such positions, these organizations place formal advertisements in the leading newspapers stating, 'Applications are invited from Indian citizen for...' to fulfil the norms, rules and regulations set by Indian law in this regard. However, it is an open secret that in many cases more favoured candidates are already around to occupy the available position. Obviously, the other aspiring persons being unaware of the real position, also apply for the same post. The treatment of these job aspirants during the 'mock' interview is saddening.

All will undoubtedly agree that interviews are conducted to assess the merit of a person for the job, not to expose his ignorance. The technical skill, awareness, outlook, temperament and above all knowledge on the particular field should be tested. On the contrary, the (biased) interviewer ask questions that do not help to reveal merit but highlight the 'imperfections' of the candidate, just to keep him at bay.

Is it ethical just to pretend during interviews? Is it a healthy practice in the overall interest of the organization and, in turn, for the country to have farcical interviews? Do interviewers ever think how much mental agony the candidate has to undergo when treated unfairly during a job interview?

When too many persons chase a few jobs, the employer is privileged because of the opportunity to pick up the best of the aspiring candidate. However, this may be true for a job in private organizations and may not be applicable for government jobs, where there are a lot of 'pushes' and 'pulls'. But should the interviewer be neutral and judicious, they could safeguard the interests of meritorious candidates.

B. K. PADHI

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Biotechnology

The write up by A. S. Rao on 'Biotechnology; What's in a name' (*Curr. Sci.*, 1996, 70, 955) makes interesting reading. Today, every biologist in India wants to be called a biotechnologist and why not. After all, as the Hungarian scientist rightly said, even pig rearing is biotechnology.

It is true that all our biotechnology institutions from the top downwards, are managed by biologists or those who have tinkered with biology. Universities and national laboratories are racing with each other to start biotechnology departments not bothering to see if they can find correct persons to man these new units. Some industries have also jumped on the bandwagon and have started bottling old wine in the new bottles. This is not all. Universities have also introduced, special admission tests to mislead the young to think that biotechnology programmes are professional programmes. No doubt, some

new techniques such as cloning, PCR, ELISA, manipulation of cells and the genetic material have been developed for use by all biologists. Mere using these techniques does not make a biologist a biotechnologist.

In the sixties, it was the craze for molecular biology and today, it is biotechnology. Like all other things, this craze will also slowly subside. This is reflected by the decrease in the number of students who take the all-India admission tests for postgraduate programmes in biotechnology. Our national funding agencies, instead of bringing together biologists of all types together, have only helped in creation of closed pockets by liberally funding programmes that were already in existence. While in some advanced countries the new techniques have helped in improving old biological processes, despite substantial support for this new area since the past two decades, nothing worthwhile has come out. All that has happened is new name plates and boards have come up all over. Perhaps, things could have been different had our managers been trained biotechnologists!

P. TAURO

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We now probably have a very large number of molecular biologists besides biotechnologists. True discrimination between a technique and a discipline or branch of knowledge is lost now. A major thrust of biotechnology involves genetic manipulations to produce a new useful entity. How does a biochemist, a botanist

CORRESPONDENCE

or zoologist working with a few enzyme estimations call himself a molecular biologist/biotechnologist/molecular virologist competent to teach and research in biotechnology, molecular genetics, molecular biology, virology or whatever? These areas require skills in a host of techniques and an interdisciplinary background is a must, as evidenced only by high quality research publications in relevant areas. Unfortunately, masquerading is common, with little regard for actual quality work, real expertise, relevancy and students' development in a discipline.

Graduate and postgraduate syllabi are decades old, though the school and pre-

university syllabi have been improved, though not necessarily in terms of practical training. Teachers rarely care to update themselves qualitatively in all respects, resulting in the destruction of the graduate and postgraduate education system.

Is there a remedy? Yes, when the sensitive, educated professionals react at qualitative level all over the country, flood with letters the Central and State ministries, the Vice-Chancellors, Director/Director-General/Chairman/Secretary of Institutions, etc., for changes in the Acts of Universities to include accountability, relevancy and competency both in academics and administration.

We should now create urgently a watchdog agency for ensuring quality education, and to control scandals. Will such people get together in large numbers in different places and demand quality? Continuous consistent lobbying should give results rapidly. The sceptres of WTO and IPR should urge us into action.

M. V. NAYUDU

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OPINION

Global errors in science: Traps of chance and prejudice

K. N. Ganeshaiah

Despite the awareness that the chances of a given ticket holder winning a lottery are dismally poor and sufficiently low enough to inspire any buying, several lakhs of people buy lottery tickets every month and lottery has been surviving as a successful enterprise. Clearly the inspiration for the buyer does not come from those millions who have lost their rupees but from that occasional lucky winner who bags several lakhs of rupees. His smiling face printed in the magazines makes news and several more millions are induced to buying tickets; though they all are mostly bound to lose, again there is another lucky winner who helps maintain the buying chain and the lottery continues to survive. Thus unlike that of the proverbial chain, the strength of this lottery chain is reflected not in its weakest length (the million losers) but in the strongest link (the lucky winner).

Surprisingly, a similar process of occasional 'discovery' seems capable of sustaining a chain of false inventions in science on a global scale. Such a possibility has recently been pointed out by Bill Amos¹ from the University of Cambridge and discussed in the columns of *Nature*^{2,3}.

Assume that a certain interesting pattern (irrespective of how one wishes to define

it⁴) has been proposed by a research worker in a symposium and that 20 of the participants are inspired to test it after their return. Even if the pattern does not exist, the laws of probability ensure that purely by chance one out of these 20 would find evidence for the pattern ($p < 0.05$). He would obviously be prompted to publish it because of his faith in the statistical significance of his experimental results and also because they are supporting what has already been held by others. The remaining 19 who could not get the 'expected' results may either feel less confident of their experimental protocols or more frequently do not find it interesting enough to publish the non-significant results. Occasionally some of them who do attempt to publish might find it hard to convince the referees against a pattern that is probably already making news.

Now assume that the paper published by the lucky scientist who got the results 'right' is read by a thousand research workers world over and that they would attempt to repeat the experiments. Again by the rules of probability, 50 of them would find the pattern to be true (< 0.05) and among them 10 would find it very highly 'significant' ($p < 0.01$). Even if only 30 of these 50 publish their results,

it leads to a chain of the publications demonstrating a pattern that indeed does not exist!!

Chance and prejudice as traps

What happens next? Does this global chain of errors continue? Or would the self-correcting mechanism of science set it right? Certainly as a preliminary step, the scientific community is likely to celebrate this news and the scientists would begin working further on this pattern that has by now become 'established'. As I argued elsewhere⁴, 'every pattern shown or demonstrated has the same effect as a miracle would have on the spread of a religion or the religious belief'. Such an error where a pattern is 'found' while it does not exist is termed Type I error in science and has the likelihood of not being noticed because of two reasons: one, the prejudice and the other the trap of chance.

First, scientists generally suffer from a prejudice of looking for only significant results—a syndrome that has been perpetuated by their incessant obsession to find patterns⁴. Consequently they have an instinctive desire to search for the existence of patterns, such that any work that does not find the pattern gets less or no

attention both by the investigators and also by the rest of the scientific community. Therefore in situations where no pattern exists, 95% of the work demonstrating no pattern is likely to remain unpublished or if published might go unnoticed. This attitude of science is an important fitness component that has contributed for its survival⁴.

Second, the scientific acceptance and approval of the pattern is innate in the statistical grammar or code the science has adopted for its own conduct: the results of the investigators who find the pattern significant has to be 'accepted' though it is likely that these might be only those statistically lucky investigators. Only a global observer with a bird's eye view of all the investigators could find that 95% of the workers have not been able to observe the pattern and have not reported this fact. But since the evaluation of the facts is not a well-coordinated global activity, rather is based mostly on individual cases, the 95% of the investigators are not going to add to the establishment (de-establishment) of 'the fact'.

There is one objection however: statistically if 50 scientists find the results significant by chance, 25 of them shall find the pattern in a positive direction and 25 in the negative direction such that the publication of both of these would end the matter and settle for a lack of pattern. But as Robert West³ points out, scientists often seek trends in one direction (perhaps positive) and hence the chain process of reporting the results that 'conform' to the already held pattern would perpetuate.

Global errors as stochastic events

The operation of global errors of this nature is in fact a pure consequence of the stochastic events reinforced by a positive feedback process. Such process has been recently realized to be important in several fields⁵. It is consequent to the way in which the probability often drives a random system towards a pattern that appears deterministic.

Consider an industrialist planning to release two models of a new product to the market. Both the models have similar efficiency and hence he has no idea which of the two would make a breakthrough. Therefore he has formulated a simple strategy for further production of the

model that customers like most: to begin with he releases just one unit each of the models and every time a unit of a model is sold in any of the outlets, he shall produce two units of that model and distribute. This way the production is directly linked to the rate of sale and the producer also guards against the overproduction of the model that does not sell well.

Accordingly, when the first customer buys one of the two models (say A), the industrialist releases two units of that model. Similarly the information on the model bought by the next customer is fed back to the producer who in turn releases two more units of that model to different outlets.

As this process goes on, and once the selling counters are flooded with the product, what would be the likely proportions of the two models in the market? Note that the marketing started off with one unit each of the models and that they cannot be discriminated by the customers as they do not have any specific differences in their customer appeal features; in fact their 'selection' of, or preference for, a model is a random process and hence would be in proportion to their availability in the market. Obviously we find it reasonable to expect that the frequencies of the models remain equal.

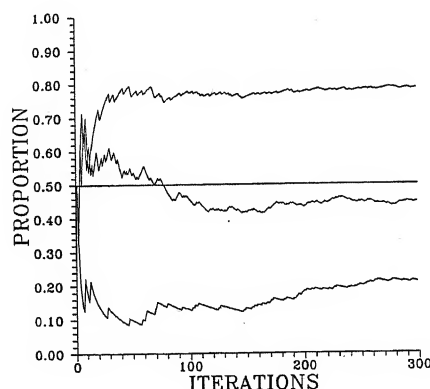


Figure 1. Frequency of red balls after every draw in three simulations of the Polya-Urn model. Note that the frequency begins with 0.5 as there are one each of red and white balls and hence it is generally expected that the frequency fluctuates around this (horizontal line). But as the process (draws; iterations) starts there will be an initial wiggle in the frequency which eventually stabilizes at some final proportion (p) in the range from 0 to 1. Summary of 2000 such simulations is presented in Figure 2.

But the mathematicians have shown that under such situations it is most likely that the market stabilizes with one of the models dominating over the other! Further, were the whole process to be repeated in another isolated island the chances are that the other model dominates the island market! In other words there is no way of predicting the fate of such models in advance.

The underlying stochastic events are well known to the mathematicians as the Polya-Urn process. The phenomenon can be visualized with a huge urn (market here) containing a red and a white ball (the models here); a ball is randomly drawn and returned to the urn with an additional ball of the same colour (as the industrialist does with the sold model) and the process is repeated. If one keeps track of the frequency of the two colours, initially, when there are just a couple of balls in the urn, the proportion of any colour shows heavy wiggles (Figure 1) because even a slight random bias in picking a particular colour can cause huge changes in the proportion. A chance favoured bias in drawing only red balls for instance, adds more red balls to the urn and increases the probability of drawing red balls further. However as the number of balls in the urn increases, this proportion converges to some limiting value p and as the urn gets filled with a large number of balls, the system becomes resistant to any random bias in picking the balls and hence gets stabilized. What is most interesting is that p can take any value between 0 and 1 (Figure 1).

I have simulated this process 2000 times and the results are plotted in Figure 2: clearly all values of p are equally

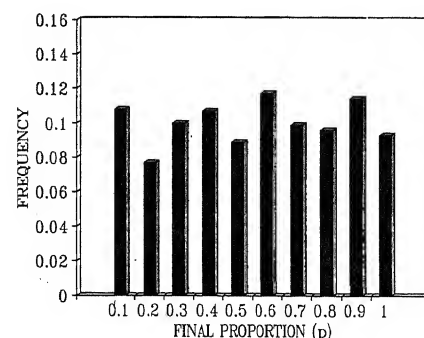


Figure 2. Frequency of the final proportions (p) of red balls in 2000 simulations. Note that all levels of p are equally probable and hence it is very difficult to predict the fate in advance.

probable! In other words, back to the marketing scenario, the extent to which a particular model dominates is purely a consequence of the history of the market and not a deterministic process. That science might frequently use these deterministic end results as the basis of investigation and attempts to probe for deterministic causes is another issue. A far more serious consequence of such stochastic process is the possible feedback chain of erroneous reports it might start off in science. As described earlier, such stochastic events based on the permissi-

bility of the statistical codes of conduct adopted by the scientists might start a chain of false discoveries that might never appear to be the global errors in science. It appears it is very imperative to evaluate the extent to which our scientific information is clouded with such global errors generated purely by chance and nurtured by the prejudices of scientific community. As Bulstrode⁶ quips in *Nature*, 'The logical conclusion is, sir, your journal may be merely noise'.

1. Amos, B., *Nature*, 1996, 379, 484.

2. Dunthorn, D., *Nature*, 1996, 380, 477.
3. West, R., *Nature*, 1996, 380, 477.
4. Ganeshaiah, K. N., *Curr. Sci.*, 1995, 68, 680-682.
5. May, R., *Nature*, 1976, 262, 646.
6. Bulstrode, C., *Nature*, 1996, 379, 765.

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COMMENTARY

A survey of Himalayan resources

David A. Spencer and Rasoul B. Sorkhabi

Himalayan geology is increasingly drawing the attention of the international geological community. In response to this 'global age of Himalayan geology', several community services and information networks have appeared in recent years. Recently, we conducted an international survey among Himalayan researchers to evaluate how they use these resources, and to find what improvements can be made to them. The survey was sent out in February 1996 to some 500 active Himalayan researchers in various countries, whose names were available to us. There was a truly international participation in this survey, reflecting the current range of researchers from many countries. The survey was also posted twice on the electronic mail network 'HimNet' (4 and 25 February 1996), which has a distribution to 580 researchers. There is a large overlap between our survey database list and the HimNet subscription list. A total of 258 replies to the survey were received, which account for nearly 40% of our mailing list. Here we briefly report the results of the survey. Although this survey is meant to assess the state of networking and information resources among Himalayan geologists, the ideas and results derived from our survey may

also be useful for development of networks and community services in other fields of geological research and scientific activity.

HimNet (Himalayan Network)

HimNet (HimNet@erdw.ethz.ch) is an e-mail Internet link for researchers working in the Himalayan region (Afghanistan, Pakistan, China, India, Nepal, Bhutan, Bangladesh and Burma). It aims to provide a rapid method of communication and dissemination of information on Himalayan geosciences. HimNet sends direct e-mails to its subscribers. Launched by David A. Spencer in August, 1994, with an initial subscription of 40 people, the number of subscribers has constantly increased over time to 580 (Figure 1).

By May 1996, 27 news packages have been sent to subscribers in 32 countries. Although predominantly related to the geosciences, Himalayan researchers in several other fields (from biology to linguistics) also subscribe to HimNet. Last year, a World Wide Web Home Page (<http://www.lehigh.edu/~inees/himnet/himnet.html>) was set up, and is maintained by Peter K. Zeitler (Lehigh University, USA), allowing access to all the back

issues of HimNet through the WWW or FTP (<ftp://ftp.dharma.geo.lehigh.edu>; User ID: anonymous; Password: your e-mail address; Directory: /pub/himnet).

Virtually all of those who responded to the survey (92%) knew about HimNet; 66% subscribed to it. As for the 34% who did not subscribe to HimNet, the lack of internet connection was cited as the main reason by them (63%). This was especially so in the Himalayan coun-

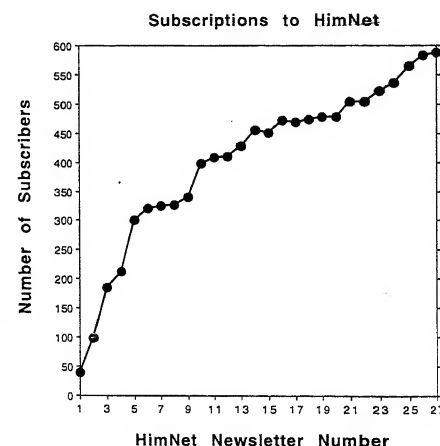


Figure 1. Subscriptions to the Himalayan Network (HimNet).

tries, where internet connections are not available, or they are too expensive (charged per page) or official permission is required to have an access to e-mail. Other reasons for not subscribing to HimNet included phobias to mailing lists, the fact that the postings took up too much disk space or simply not having time to read it.

The most popular features on HimNet were the Latest Himalayan Papers (91%), Latest Himalayan books (85%) and Conference Announcements (82%). It seems that subscribers use HimNet mainly as source of information, rather than as a medium for discussion. This was also clear from the fact that nearly two thirds of those who subscribed (62%) had never made a contribution to HimNet. Nevertheless, 96% of the subscribers thought that HimNet is a valuable research tool; 61% rated it as excellent and 34% as good.

Himalayan Notes

Himalayan Notes is an international news bulletin on the earth and environmental sciences of the Himalaya-Tibetan region. Founded in early 1993 by Rasoul B. Sorkhabi, it is published twice a year in March and September. *Himalayan Notes* attempts to foster up-to-date information on south-central Asia's geosciences, provide a platform for discussions and exchange of ideas and act as an informational bridge between Himalayan and foreign scientists. It also aims to draw people's attention to the unique geology, environmental problems and global significance of the Himalaya and Tibet. Geoscientists, and others working or interested in the Himalayan-Tibetan region, have subscribed to *Himalayan Notes*. Moreover, some institutions and libraries in Europe, America and the Himalayan countries subscribe to this newsletter. It is also exchanged with some Himalayan periodicals on a regular basis. Regular sections in each issue of *Himalayan Notes* include: In brief (meetings and other short news items), In press (coverage of Himalayan geology in the press), Research line (latest papers, maps, theses, and books), Institutions, Book reviews, Conference reports, Travel accounts and Profiles of Himalayan researchers. At present, *Himalayan Notes* is coordinated by a group of regional editors (in Europe, India, Pakistan, and

Nepal) and a managing editor in the USA.

Nearly 78% of the people surveyed were aware of *Himalayan Notes*, although only 26% subscribed to it. This is mainly because the sending of a small subscription fee for non-USA subscribers was difficult (the overseas bank charges would cost more than the subscription fee!). This problem could be resolved by accepting payment with credit cards. The most popular features of *Himalayan Notes* were the Research Line (read by 98% of subscribers), Conference reports (89%), Book reviews (89%), In brief (86%) and In press (82%). Only half of those subscribed read the travel accounts (52%) and articles about the institutions (48%). Therefore, the subscribers used *Himalayan Notes* mainly as a source of research information, rather than a magazine with feature articles. Similar to HimNet, 57% of the subscribers had never made a contribution to *Himalayan Notes*. Nevertheless, 93% of them thought that it was a valuable information tool and 75% were content with it being published twice a year (18% suggested that the frequency of publication should be increased to four times a year). Although *Himalayan Notes* is predominantly seen as a source of research information, a clear 95% agreed that social issues and cultural aspects of the Himalayan region may be addressed in *Himalayan Notes* from a scientific standpoint and with regards to the natural environment. Most subscribers seem to be satisfied with *Himalayan Notes*, rating it good (68%) or excellent (27%).

The Himalaya-Karakoram-Tibet workshops

Initiated by Michael P. Searle in 1985, the first Himalaya-Karakoram-Tibet (H-K-T) workshop was held in Leicester, Great Britain. Subsequent H-K-T workshops were held in Nancy, France (1986); London, Great Britain (1987); Lausanne, Switzerland (1988); Milan, Italy (1990); Grenoble, France (1991); Oxford, Great Britain (1992); Vienna, Austria (1993); Kathmandu, Nepal (1994); Ascona, Switzerland (1995); and Flagstaff, USA (1996). Proceedings volumes resulting from the H-K-T workshops form an excellent source of up-to-date geological information. These workshops continue to be one of the main forums for pres-

entations on all aspects of Himalaya-Karakoram-Tibet geology.

Virtually everyone who responded to the survey (98%) knew about the H-K-T workshops and 57% had attended at least one of them. The majority of people (89%) who had attended an H-K-T workshop had made a presentation (either oral or poster), indicating that the workshops are clearly seen as a 'participation' event rather than a 'spectator' event. The workshops are also used as an opportunity to meet colleagues (91%), listen to lectures (94%) and discuss Himalayan issues (91%). Although only 56% were interested in the publication of proceedings volumes resulting from the workshops, over 72% stated that they had referred to at least one of the papers published in the past H-K-T proceedings volumes. Nearly half (43%) of those who had attended the workshops considered them too expensive. Nevertheless, 82% considered that the H-K-T workshops mark an important event in their research calendar.

Perhaps one of the most surprising results was that nearly two thirds (65%) suggested that the workshops be held once every 2 years rather than every year (supported by 32%). There were even a few suggestions for holding the workshop every 4 years! The main reason for reducing the frequency of the workshops was that 'because they are too frequent, there is a tendency for delegates to repeat the previous year's talk'. A suggestion was also given that 'because every country is expensive for someone from somewhere, the workshop should (as has been in the last 3 years) be rotated every year to different continents so that all would have a chance to attend. This is only fair'. To date, nine of the eleven H-K-T meetings have been held in Europe. Overall, 57% thought the workshops have been 'good' and 43% rated them as 'excellent'.

International Society of Himalayan Geoscientists

Recently, Rasoul Sorkhabi has brought forward the suggestion that there is a need for the creation of an International Society of Himalayan Geoscientists because, as history shows, scientific disciplines are better developed when their respective communities have become organized, with efficient ways of exchanging information, regular publications and

meetings. 72% of those who responded to the survey felt that it was necessary to establish an International Society for Himalayan Geoscientists. Such a society should mainly be involved in organizing the Himalaya-Karakoram-Tibet workshops (93%), promoting the importance of research in the Himalayan regions (88%) and representing the scientific community (76%). Its other goals may include publishing a newsletter (75%), publishing a research journal (68%) and running an electronic network system (82%). Although not seen necessarily as a 'funding agent', 76% (especially those from the Himalayan countries) suggested that such a Society should try to arrange funds from international or Western world funding agencies to enable students and researchers from the Himalayan countries to attend international meetings. Half (53%) of those who supported the idea of founding an International Society for Himalayan Geoscientists indicated that they were willing to get involved in its establishment.

However, there were also some arguments against the formation of the Society. For example: 'Such organizations divert energy away from research'; 'Official organizations actually hinder cooperation, especially between young and old scientists'; 'No need for another society. A formal organization would not improve our situation unless a lot of effort and time is devoted to it.' Most of those who were against the formation of a Society were the more established and experienced Himalayan geologists from Europe or the USA, while virtually all of the researchers from the Himalayan countries supported the idea.

Summary and concluding remarks

(1) Overall, the Himalayan research community appreciate the importance of networks and information resources available to them; however, only a minority of them actively contributed to the community services.

(2) Both HimNet and *Himalayan Notes* are relatively newcomers to the scene of Himalayan research. HimNet is free of charge, while *Himalayan Notes* requires subscription fees to cover the cost of printing and postage. This, and the fact that HimNet is a faster method of communication are the main reasons for the large disparity between the numbers of their subscribers. Nevertheless, both of these media enjoy large support. The subscribers use them largely to obtain information rather than for discussion. Since both HimNet and *Himalayan Notes* are produced by volunteer efforts of several individuals, their future activity cannot be ensured unless they are run by some commercial body or rotated voluntarily among active Himalayan researchers. The establishment of an International Society for Himalayan Geoscientists would provide suitable channels for such volunteer efforts.

(3) The Himalaya-Karakoram-Tibet workshops are seen as the main gathering opportunity for most Himalayan researchers. Nevertheless, the majority of the surveyed people thought that these annual workshops are too frequent. Although the idea of holding the H-K-T workshops every two years needs to be closely examined, it should be noted that over the past decade, these annual meetings have become increasingly popular and have produced a large amount of geological publications on the Himalayan region (few tectonic regions and geological workshops have been so prolific).

(4) Most researchers support the idea for the creation of an International Society of Himalayan Geoscientists. Nevertheless, to succeed it would require a great deal of time and energy. As history shows (for example, the Geological Society of London was founded in 1807 by George B. Greenough; the Geological Survey of Great Britain during the 1830s mainly through the efforts of Henry De la Beche; the Royal Geographical Society in 1830 under the leadership of Sir Roderick Murchison and the US Geological Survey in

1879 by Clarence King and John Wesley Powell) most of the scientific and professional societies were founded through the cooperation of a few pioneer, like-minded individuals, not necessarily the consensus of a whole community. Indeed, the majority support of the Himalayan research community for founding a Society is a very positive sign of its success. Through determination and devotion of founders during the early years of a Society and providing useful services, scientific societies have filled a niche among their communities and succeeded. There is no reason to believe that the establishment of the suggested International Society of Himalayan Geoscientists should be any different from past historical experiences. Opportunities for such ventures are open to all Himalayan researchers in all countries. There are already several networks and resources, such as HimNet, *Himalayan Notes* and the H-K-T workshops, as well as other specialized periodicals such as the *Journal of Himalayan Geology* (published semi-annually by the Wadia Institute of Himalayan Geology, India) and the *Himalayan Research Bulletin* (two semi-annual periodicals in North America and Europe which focus on social sciences). Although all these resources can form vital elements of any international society, they are currently scattered mini-islands; they can better develop under an umbrella of an international society with high scientific and democratic standards.

ACKNOWLEDGEMENTS. We would like to thank all those who responded to the Survey of Himalayan Resources.

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Optical astronomy using radio techniques: Imaging Capella with unprecedented resolution

P. Venkatakrishnan

Radio astronomers fully vindicated the oft-quoted maxim that necessity is the mother of invention when they went about the task of imaging celestial radio sources. What may seem to be a trivial job in optics, e.g. imaging the sun on to a white screen and looking at the sunspots, would require a radio 'lens' or 'mirror' that is over 100 m wide. Likewise, a 10 km wide lens would be needed to form an image of Jupiter in microwave radiation. This is because electromagnetic waves bend around obstacles (the phenomenon of diffraction) and fail to come to an exact focus. This fuzziness increases with the wavelength of the radiation. Light waves, being a million times smaller than microwaves, can be 'tamed' by lenses that are a million time more compact than the sizes mentioned above. Faced with this immense problem, radio astronomers since the fifties have followed the Nobel prize winning lead of Martin Ryle at Cambridge. They build up their images using a collection of smaller antennas distributed over large areas. The signals from each pair of antennas are multiplied together and averaged in time, yielding a quantity called the correlation. The separation of the antennas expressed in multiples of the wavelength is called the spatial frequency. The set of correlations for various spatial frequencies are then fourier transformed (a mathematical operation that does a sort of book-keeping on the number of waves present in a pattern of signals). The resulting function gives the brightness distribution of the source, as a function of the angular position. The theorem connecting correlations in the wavefront, with brightness distribution on the source, is called the van Cittert-Zernike theorem and was developed for optics! Formally, this technique of imaging is called aperture synthesis.

There is nothing in principle that prevents optical astronomers from using this technique (although it seems a somewhat round about way of imaging as compared to more conventional ways). The correlation is obtained by combining the beams from the two apertures and forming an interference pattern (as did the great

optical physicist Michelson when he measured the diameter of some stars at the turn of the century). The larger the correlation, the deeper will be the modulation in the interference fringes. In practice, several factors inhibit the realization of this method. The path difference between the two apertures must be shorter than the longitudinal coherence length, otherwise the fringes will get washed out. This is the length occupied by that number of waves which is equal to the ratio $\lambda/\Delta\lambda$, which is the number of bandwidths present in one wavelength. For example, the coherence length is a mere 2.5 μm for a 100 nm bandwidth centered around 500 nm. The mechanical tolerance of the experimental setup is restricted to micrometre accuracies over the baselines (separations of antennas) that are used. If one wants to escape this constraint by using a very small bandwidth, then one will need to spend a lot of time to collect the required number of photons to achieve decent signal to noise ratios. However, the fluctuations in the atmosphere (that produce twinkling of starlight) allow only about 10 milliseconds to obtain the interference pattern. If one thinks that having large apertures would do the trick, then the spatial extent over which the standard deviation of the atmospheric fluctuations are within a reasonable limit (say, a quarter of the wavelength) is only 10 cm. Thus, having apertures larger than this limit does not help. So aperture synthesis at optical wavelengths is an extremely demanding enterprise and would interest the astronomers only if they need angular resolutions much beyond the diffraction limits of large single telescopes. A few groups of astronomers have been able to interfere light from two separated telescopes and use the depth of modulation of the fringes to resolve binary stars that are separated by a few hundredths of arcseconds. The prospect of producing a genuine interferometric image seemed bleak. But not anymore. For this feat has recently been achieved by John Baldwin and coworkers at Cambridge, UK (*Astronomy and Astrophysics*, 1996, 306, L13-L16).

The extra requirement to form an image

is the knowledge of the fourier phase of the target source. The fourier transform of a symmetrical object is a real function which comes with a plus or minus sign. Asymmetric objects yield complex fourier transforms needing a phase angle anywhere between 0 and 360 degrees for full specification. The fourier phase (or visibility phase) is then the argument of the complex fourier transform. The modulus of the transform is called the fourier amplitude, or visibility amplitude. It represents the contrast of variations in the object at the given spatial frequency. The phase represents the morphology, or positional information of the variations at that spatial frequency. For example, an unresolved object at the centre of the field would continue to have undiminished visibility amplitude of unity at the largest separation of the antennas (or telescopes), corresponding to the largest spatial frequency. The phase would be zero degrees all through. A resolved object would have an amplitude that goes to zero at a spatial frequency corresponding to the inverse of the angular size of the source, while the phase switches from 0 degrees to 180 degrees at this spatial frequency. Knowledge of phase is crucial for imaging, since it provides basic information on the morphology of the object. For example, when the fourier amplitudes of a binary source (shown in Figure 1 a) are randomized, while retaining the phases, the object in Figure 1 b is obtained which is still a binary, albeit with different contrast. On the other hand, if the phases are randomized, keeping the amplitudes intact, the resulting image (Figure 1 c) is scarcely recognizable. Actually, what is needed is the relative phase between two spatial frequencies. Because of turbulence, it is impossible to 'freeze' one phase. So we require simultaneous measurements of three phases, hence we need at least three antennas or telescopes. If the turbulence introduces a random phase in antenna 2, it has opposite effects on baselines 12 and 23. The sum of phases on 12, 23 and 31—the so-called closure phase, is thus an intrinsic property of the object with random properties of the medium cancelling out.

RESEARCH NEWS

The COAST (Cambridge Optical Aperture Synthesis Telescope) instrument developed by John Baldwin and his group has four telescopes, of which three were used to image the star Capella. These telescopes are simple in design. The object is tracked by siderostats (flat mirrors rotating about an axis parallel to earth's rotation axis and inclined suitably to send the reflected beam along a fixed direction). These siderostats feed horizontal telescopes. This design has the great advantage that long moving parts are avoided, thereby achieving the stringent limits of mechanical accuracy set by the longitudinal coherence length. Light from each telescope is brought along evacuated pipes into a thermally insulated interference station (actually a grass covered bunker!). Pupil plane interferometry is employed rather than interference in the image plane. The advantage is that the width of the fringes can be controlled independent of the focal length of the imaging system. This is particularly useful for photon starved observations. In the COAST setup, the images of the interfering pupils are kept parallel, so that there is uniform illumination at the interfering plane. The interference is then detected by introducing a periodic path delay of about $50\text{ }\mu\text{m}$ sweep in each arm to produce a corresponding modulation

in the combined light. This modulation is detected by an avalanche photodiode operating as a photon counter. The beauty of the arrangement is in the modulation of the fringe pattern of each pair of telescopes with a distinct periodicity, viz. 238, 477 and 715 Hz respectively. So, a single detector can record all the fringe visibilities, which are encoded in distinct temporal frequencies. The triple product of the three complex visibilities, corresponding to the three baselines can thus be conveniently recorded. The argument of the resulting complex number is the closure phase. Once the closure phase is known, the radio astronomers know how to reconstruct images from the visibility amplitudes and closure phases of a set of sparsely distributed baselines. The COAST experiment used interspersed observations of a single star to calibrate the visibility amplitudes.

Because of incomplete coverage in the spatial frequency domain, the reconstructed images will have many 'side lobes', which are carefully removed by an algorithm called CLEAN, which is very popular among radio astronomers. The projections of the three telescopes on to the sky plane move around with the rotation of the earth and a nice spatial frequency coverage can be obtained in this way. Two images of Capella were

obtained for two epochs separated by 15 days. The relative positions of the components lie on an orbit agreeing with that predicted by other measurements. The separation of the binary is in the region of 50 milliarcseconds, while the position angle changed by about 50 degrees during the 15 day interval (see Figure 2).

Apart from the fact that this is the first genuine optical interferometric imaging of a celestial object with separated telescopes, it also provides an exciting peek into the future of optical astronomy. Conventional astronomers seek to build larger and larger telescopes to look at very faint objects. Eventually, when the mechanical problems of large telescopes become unsurmountable, they will have to fall back upon the concept of telescope arrays which Antoine Labeyrie, John Davis, Michael Shao, John Baldwin and their ilk have courageously tried to advance. Of course, it has not been easy for the COAST group (see *Physics Today*, April 1996, pp. 17-18). The project meth-

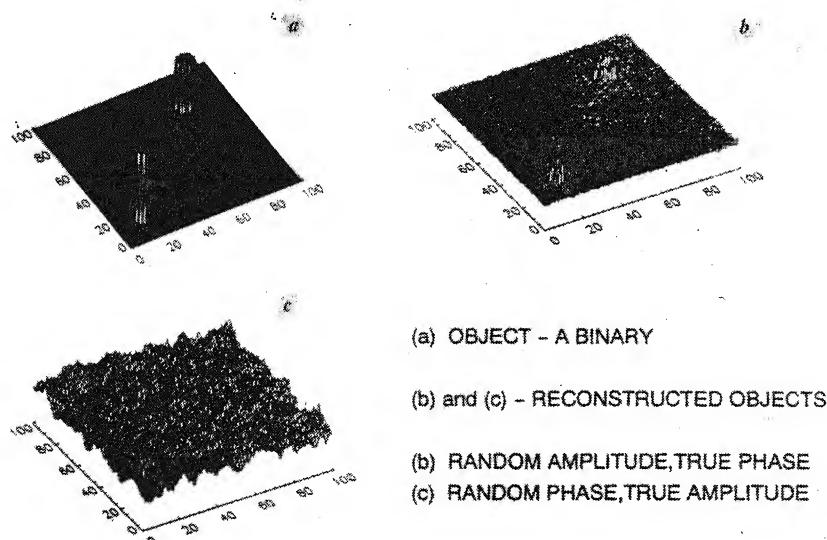


Figure 1 a-c. This figure shows the importance of visibility phase. The object of Figure 1 a was Fourier transformed. The Fourier phases were first left untampered. The Fourier amplitudes were replaced by random numbers, with total power being conserved in the Fourier domain. The reconstructed image is shown in (b). Next the Fourier amplitudes of the original object were retained while the Fourier phases were replaced by random numbers. The result of reconstruction is shown in (c). (Generated by R. Sridharan, Indian Institute of Astrophysics.)

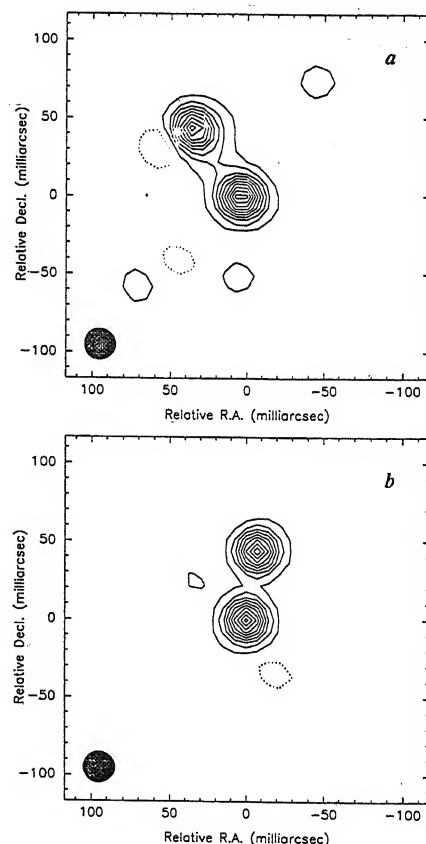


Figure 2. Image reconstruction of Capella from data obtained on the nights of the 13th (a) and 28th (b) September 1995. [Reproduced with permission from J. E. Baldwin *et al.*, *Astron. Astrophys.*, 1996, 306, L13-L16]

odology adopted by Baldwin is worth special attention, especially for younger persons embarking on new techniques. He started with experimenting on a few masks placed over a single telescope and gradually worked his way up to the current achievement. The paper which

reported this achievement has a large number of authors from several institutions—a model for successful cooperation that merits special mention. We can expect several new discoveries when the sensitivity and resolution of this technique improve, and when the several other con-

tenders in this game achieve similar successes.

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SCIENTIFIC CORRESPONDENCE

Volatile oil constituents and wilt resistance in cumin (*Cuminum cyminum* L.)

Cumin cultivation has received a serious threat from wilt disease which devastates the total standing crop. Screening of lines against cumin wilt under artificial/field conditions indicated that UC-198, UC-199 and RZ-19 have shown fairly good tolerance to wilt¹. Different entries of cumin were evaluated for volatile oil contents and correlated with wilt incidence. To understand the role of volatile oil in disease resistance at the molecular level, the volatile oil was fractionated on GLC and correlation between volatile oil constituents and wilt resistance was determined.

Fourteen entries of cumin were grown at Agriculture Farm of S.K.N. College of Agriculture, Jobner under All India Coordinated Varietal trial. The volatile oil contents from these entries were evaluated using Clevenger apparatus². The volatile oil was made moisture-free by using anhydrous sodium sulphate and

stored in a glass vial. These samples were subjected to gas chromatographic examination as described earlier³. The identity of the constituents was ascertained by comparison of relative retention time with authentic standards. The percentage of oxygenated compound and hydrocarbons was recorded.

The negative correlation between volatile oil contents and wilt incidence (Figure 1) suggests that the variety with high volatile oil contents is less prone to wilt which was not true in all cases. The anti-microbial property of volatile oil was also reported by other workers^{4,5}. Earlier we reported that the growth of wilt pathogen, *Fusarium oxysporum* was inhibited in presence of volatile oil of cumin. The effectiveness of inhibition varies from variety to variety⁶. To understand it at the molecular level, the main components of volatile oil of some varieties of cumin were determined. Volatile oil of cumin

consists of a mixture of hydrocarbons (terpenes, sesquiterpenes, etc.) and oxygenated compounds (alcohols, ester, ether, aldehydes, ketones, lactones, phenols, etc.). Of these, the oxygenated compounds are the principal odour carriers, although the terpenes and sesquiterpenes too contribute to some degree to total odour and flavour. These components are separated on GLC on the basis of their partition coefficient. The hydrocarbon components and oxygenated compounds are having marked difference in the retention time due to polarity differences. The main constituents of volatile oil of cumin are cuminaldehyde, cuminyl alcohol, terpenes, p-cymenes, pinenes, etc. The first two components are oxygenated compounds and the last three are hydrocarbon compounds. So cumin oil has two types of

Table 1. Volatile oil constituents of cumin and relative wilt resistance

Entry	Volatile oil constituents (%)		Ratio of a : b	Relative wilt resistance (0-9 scale)
	a	b		
UC-199	76	21	3.61	1-2 (R)
UC-198	55	38	1.44	1-2 (R)
UC-19 mnt	42	54	0.77	2-4 (MR)
UC-218	34	61	0.55	4-5 (MR)
RZ-19	30	67	0.44	4-5 (MR)
RS-1	30	68	0.44	7-8 (HS)
UC-208	28.4	71.5	0.397	4-6 (MR)
Local	16	82	0.19	9 (HS)

a = oxygenated components; b = hydrocarbon components. R = resistance; MR = moderately resistance; HS = highly susceptible.

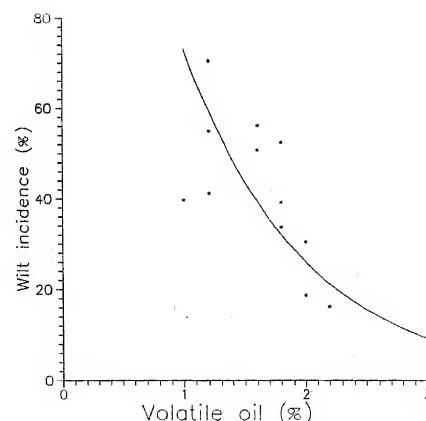


Figure 1. Relation between volatile oil and wilt incidence in cumin. The line represents the regression line ($Y = \exp(-1.03164x) 205.276$).

SCIENTIFIC CORRESPONDENCE

components, i.e. oxygenated compound and hydrocarbon compound. The percentage of volatile oil constituents and the ratio of oxygenated components : hydrocarbons were found correlated with wilt resistance (Table 1); the varieties having high ratio of oxygenated components : hydrocarbons are more resistant to wilt compared to those with lower ratio. Thus oxygenated compounds, i.e. cuminaldehyde and cuminyl alcohol appear responsible for resistance to wilt.

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Dated 24 July 1996

REGISTRAR

Heavy rainfall during monsoon season: Point and spatial distribution

P. R. Rakhecha and P. R. Pisharoty

The daily rainfall records of about 5000 stations in India, during the monsoon periods of several decades, have been examined for occurrence of heavy rainfall. The point values of (i) the highest annual rainfalls, (ii) the highest rainfalls of one, two, and three day durations, and (iii) the highest hourly values, are presented in the form of tables and charts. The highest depth-area-duration values for twelve storm-centres are also given.

AN important feature during the Southwest and Northeast monsoon seasons of India is the occurrence of heavy rainfall associated with certain meteorological situations all over the country. Persistent copious rains throughout a season are associated with orographic lifting of moisture-laden winds, heavy rains for periods of days with cyclonic storms and the short period heavy falls with intense thunderstorms. The Western Ghats is an excellent example of an area where heavy rainfall occurs due to orographic effects. Rainfall caused by a depression is as high as 40–80 cm per day. Many stations have recorded their mean annual rainfall in a single day.

A great proportion of variability of rainfall in India is related to the occurrence and intensity of extremely heavy rainfall events and as such there is a need to know the magnitudes of heavy rainfall events over different parts of the country. Discussions on heavy rainfalls are also of great importance in the designing of water projects. The spatial distribution of point and areal values of highest rainfalls over the Indian region on the basis of historical rainfall data of a large number of stations, are summarized in this paper.

measurement sites were established throughout the country. At present there are about 5480 rainfall stations spread all over the country. The daily rainfall data of these stations are available in digital form at the National Data Centre of the India Meteorological Department, at Pune.

Causes of heavy rainfall

Heavy rainfalls occur over different parts of the country and are associated with:

- i) Formation and subsequent movement of cyclonic disturbances across the country
- ii) Orographic lifting of moisture air as it rises along the slope of a mountain barrier across the air stream
- iii) Breaks in the monsoon, when the rainfalls are confined to the Himalayas and the Indian regions close to it.

The cyclonic disturbances are low pressure systems in which the associated wind circulates in a counter clockwise direction in the Northern Hemisphere. The

Rainfall stations in India

Rainfall measurement in India was started towards the end of the 18th century. The first recorded data were obtained at Calcutta in 1784 followed by observations at Madras from 1792, Bombay from 1823 and Simla from 1840. However, the recording of rainfall at a large number of stations was started from the middle of the 19th century. The need for increasing the networks of rainfall sites for hydroclimatic purposes was felt after 1947 and, as a result, extensive networks of rainfall

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Table 1. Highest annual rainfalls

Station	State	Amount (cm)
Amboli	Maharashtra	748
Agumbe	Karnataka	828
Bhagamandala	Karnataka	603
Buxa	West Bengal	532
Cherrapunji	Meghalaya	1087
Denning	Tripura	532
Gaganbavada	Maharashtra	621
Mahabaleshwar	Maharashtra	623
Matheran	Maharashtra	517
Mawsynram	Meghalaya	1141
Makut	Karnataka	506
Neriamangalam	Kerala	588
Peermade	Kerala	517
Pulingoth	Karnataka	594

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criteria for describing the cyclonic disturbances over the Indian sea areas are as follows:

System	Wind speed in circulation
Low pressure	< 20 knots
Depression	20–33 knots
Cyclonic storm	34–47 knots
Severe cyclonic storm	> 48 knots

When the maximum wind in the circulation reaches 64 knots or more, the system is called a severe cyclonic storm with a core of hurricane winds.

During the SW monsoon period the cyclonic disturbances (mainly depressions with associated winds of 20–33 knots) form over the north of the Bay of Bengal to the north of 18°N and move in a northwesterly direction across the country. Depending upon the tracks

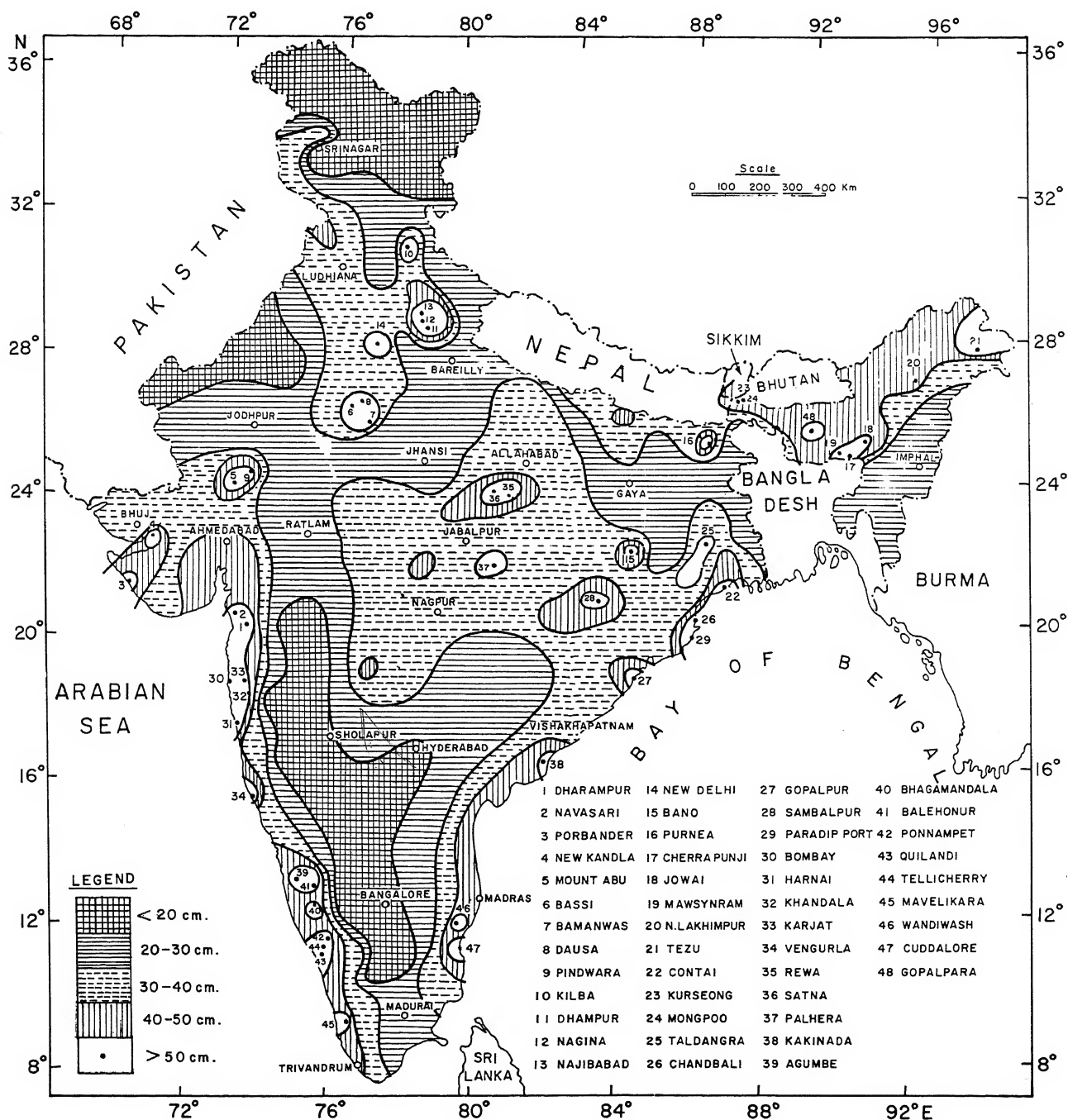


Figure 1. Highest rainfall for 1-day duration (period 1875–1982).

of these disturbances, heavy rainfall occurs in the regions which are exposed to these disturbances. Rainfall caused by such depressions can range from 40–80 cm per day. The maximum rainfall recorded in the plains during these disturbances are 90 cm in one day at Purnea in Bihar and 99 cm in one day at Dharampur in Surat district of Gujarat. The average annual frequency of monsoon depressions are 7, of which about 1 occurs

in June and two each in July, August and September. During the active monsoon the strengthening of the Arabian Sea current results in heavy rainfall along the west coast of the peninsula and on the Western Ghats. When a depression forms in the Bay of Bengal often the Arabian Sea monsoon current is strengthened; it causes heavy rainfall over the Western Ghats; due to orographic lifting of the moisture-laden winds.

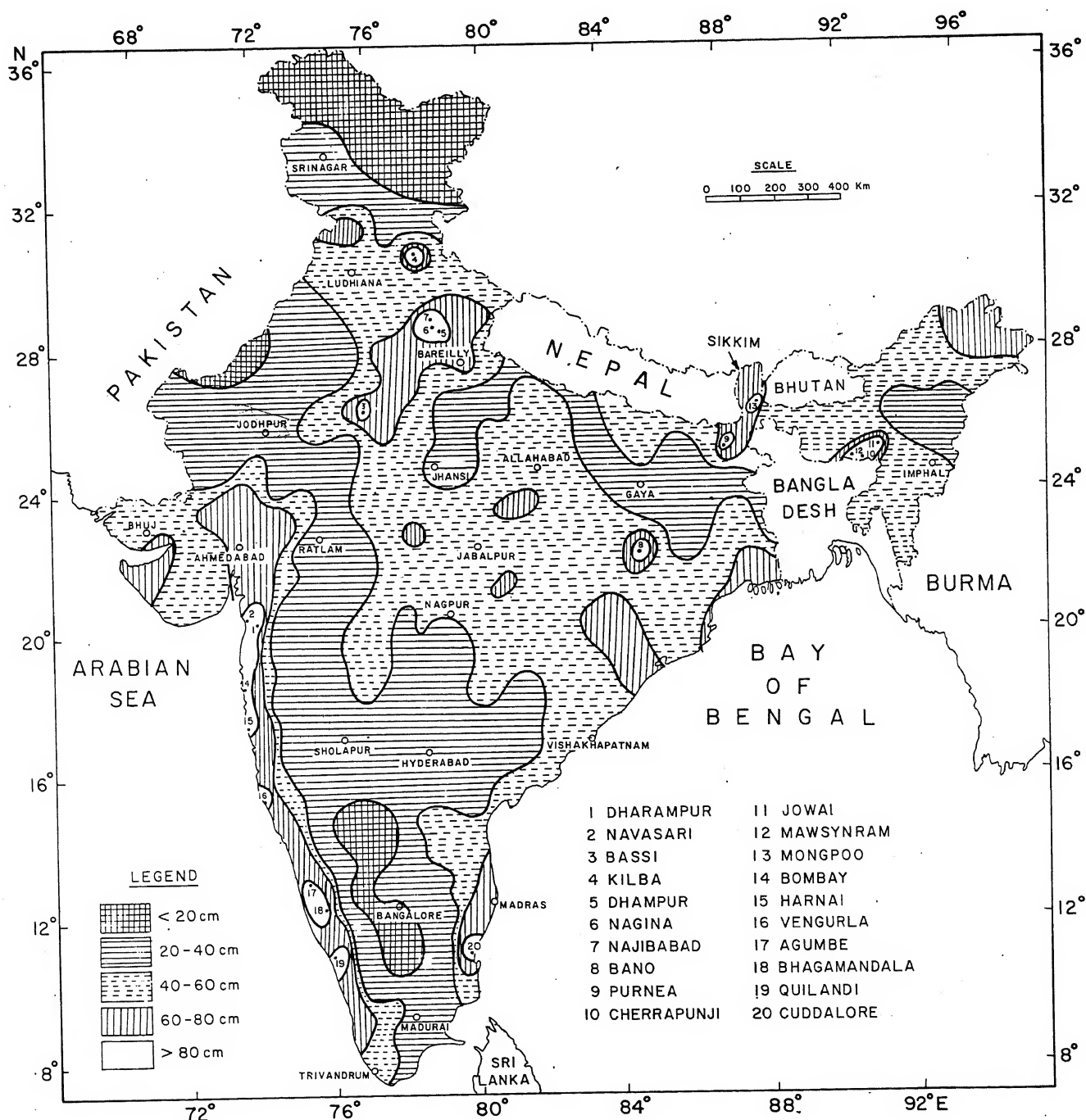


Figure 2. Highest rainfall for 2-day duration (period 1875–1982).

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Rainfall during the monsoon season is not continuous but alternates with active and break monsoon conditions. During a 'break' the seasonal monsoon trough of low pressure shifts northwards from its normal position to the foothills of Himalayas where heavy rainfalls occur.

During the pre-monsoon and post-monsoon months, tropical storms form in south Bay of Bengal and Arabian Sea. Many of these storms move inland and cause heavy to very heavy rainfall along and near their tracks over the southern parts of India.

The magnitude and frequencies of the heavy rainfalls, however, differ widely because of variations of physiography and atmospheric features.

Highest annual rainfalls

The data on annual rainfall, from about 4000 stations, show that there are about 14 stations in the country where mean annual rainfall is 500 cm or more (Table 1). Of these 14 stations, 4 stations (Buxa, Cherrapunji,

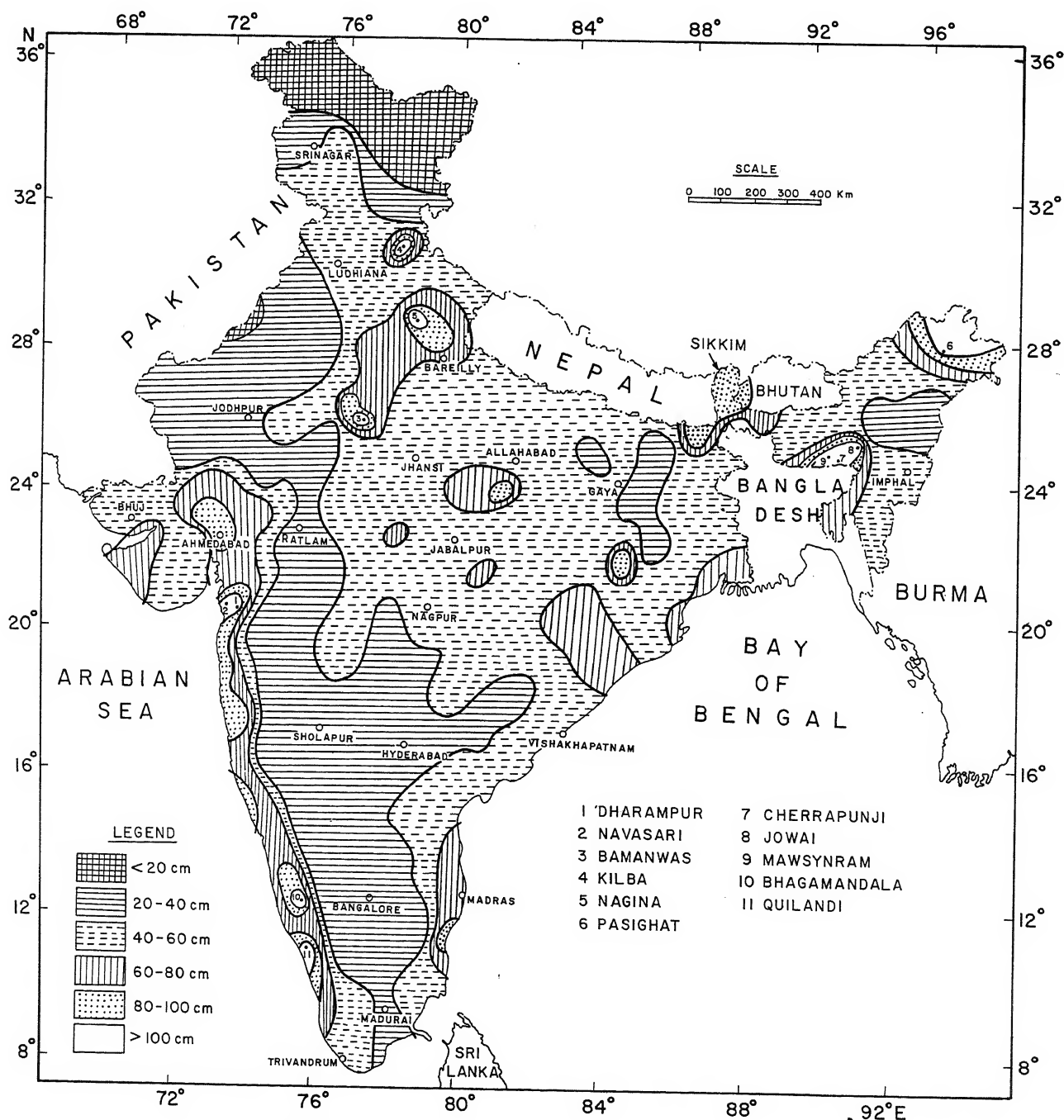


Figure 3. Highest rainfall for 3-day duration (period 1875-1982).

Table 2. Highest recorded point rainfalls (cm) for 1, 2 and 3-day durations (1875 to 1982)

Station	State	Height (m)	1-Day	2-Day	3-Day	Date
Mawsynram	Meghalaya	1401	99	143	201	July 1952
Cherrapunji	Meghalaya	1313	104	165	224	June 1876
Bhagamandala	Karnataka	876	84	136	136	July 1924
Ponnampet	Karnataka	857	52	61	67	July 1965
Agumbe	Karnataka	659	62	93	95	July 1963
Satna	Madhya Pradesh	549	54	58	61	June 1882
Khandala	Maharashtra	539	52	67	73	July 1958
Bassi	Rajasthan	351	56	84	85	July 1981
Rewa	Madhya Pradesh	286	77	77	82	June 1882
Dhampur	Uttar Pradesh	258	77	99	99	Sept. 1880
Barnanwas	Rajasthan	252	51	76	103	July 1981
Nagina	Uttar Pradesh	250	82	104	104	Sept. 1880
Najibabad	Uttar Pradesh	240	72	98	98	Sept. 1880
Karjat	Maharashtra	107	61	67	73	July 1958
Dharampur	Gujarat	38	99	126	145	July 1941
Gopalpur	Orissa	17	51	65	70	Oct. 1954
Porbandar	Gujarat	12	51	62	66	Sept. 1977
Cuddalore	Tamil Nadu	12	57	82	95	May 1943
Bombay	Maharashtra	11	57	80	88	July 1974
Vengurla	Maharashtra	9	53	82	88	June 1958
Kakinada	Andhra Pradesh	8	50	53	57	June 1941
Quilandi	Kerala	8	91	109	113	May 1961

Table 3. Depth-area-duration values (cm) of extreme rainstorms in India

Rainstorm date	Storm centre	Area affected	Duration	Area in 100 km ²					
				0	1	10	50	100	200
17-18 Sept. 1880	Nagina	Uttar Pradesh	1	82	82	78	63	52	40
			2	104	103	99	87	77	62
20-22 Sept. 1900	Serampore	West Bengal	1	44	43	41	36	33	28
			2	73	72	67	58	52	44
			3	83	82	78	69	62	52
19-21 Sept. 1926	Bichhia	Madhya Pradesh	1	36	36	35	33	30	26
			2	65	65	63	57	53	47
			3	83	82	81	76	71	62
1-3 July 1930	Wani	Maharashtra	1	36	36	31	24	22	19
			2	71	70	58	40	33	28
			3	77	76	66	47	39	35
1-3 July 1941	Dharampur	Gujarat	1	99	97	85	65	54	43
			2	127	126	118	97	83	66
			3	145	143	134	117	105	86
17-19 May 1943	Vanur	Tamil Nadu	1	42	41	37	29	25	21
			2	72	72	69	55	46	37
			3	95	95	91	73	61	49
3-5 Oct. 1955	Batala	Punjab	1	50	47	45	40	35	29
			2	72	70	64	56	51	44
			3	72	71	67	59	53	47
1-3 Oct. 1961	Sheikhpura	Bihar	1	37	37	36	32	28	23
			2	55	54	53	49	44	35
			3	58	57	57	54	50	42
28-30 Sept. 1964	Atmakur	Karnataka	1	24	23	23	22	21	19
			2	44	43	32	27	25	22
			3	62	61	51	38	34	30
13-15 July 1965	Nizamsagar	Andhra Pradesh	1	51	49	39	25	20	16
			2	54	52	41	27	23	20
			3	60	57	45	30	27	23
18-20 July 1981	Bassi	Rajasthan	1	56	56	54	45	37	27
			2	84	83	76	62	52	40
			3	97	95	85	71	61	48
28-30 Aug. 1982	Bijapur	Orissa	1	52	52	51	45	38	30
			2	70	70	69	65	59	50
			3	88	88	84	74	66	55

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Denning, Mawsynram) are located in northeast India and the other 10 stations are located in the Western Ghats of Peninsular India. Apparently, very high annual rainfalls occur in the hilly regions. Mawsynram in the Khasi hills and Agumbe in the Western Ghats of south India have received the highest annual mean orographic rainfall of about 1141 cm and 828 cm respectively.

Highest daily rainfalls

Heavy to very heavy rainfall for periods of days occurs associated with the movement of cyclonic disturbances from the Bay of Bengal and the Arabian Seas over India. The daily rainfall data for about 300 stations for the period 1875 to 1982 were used to determine the

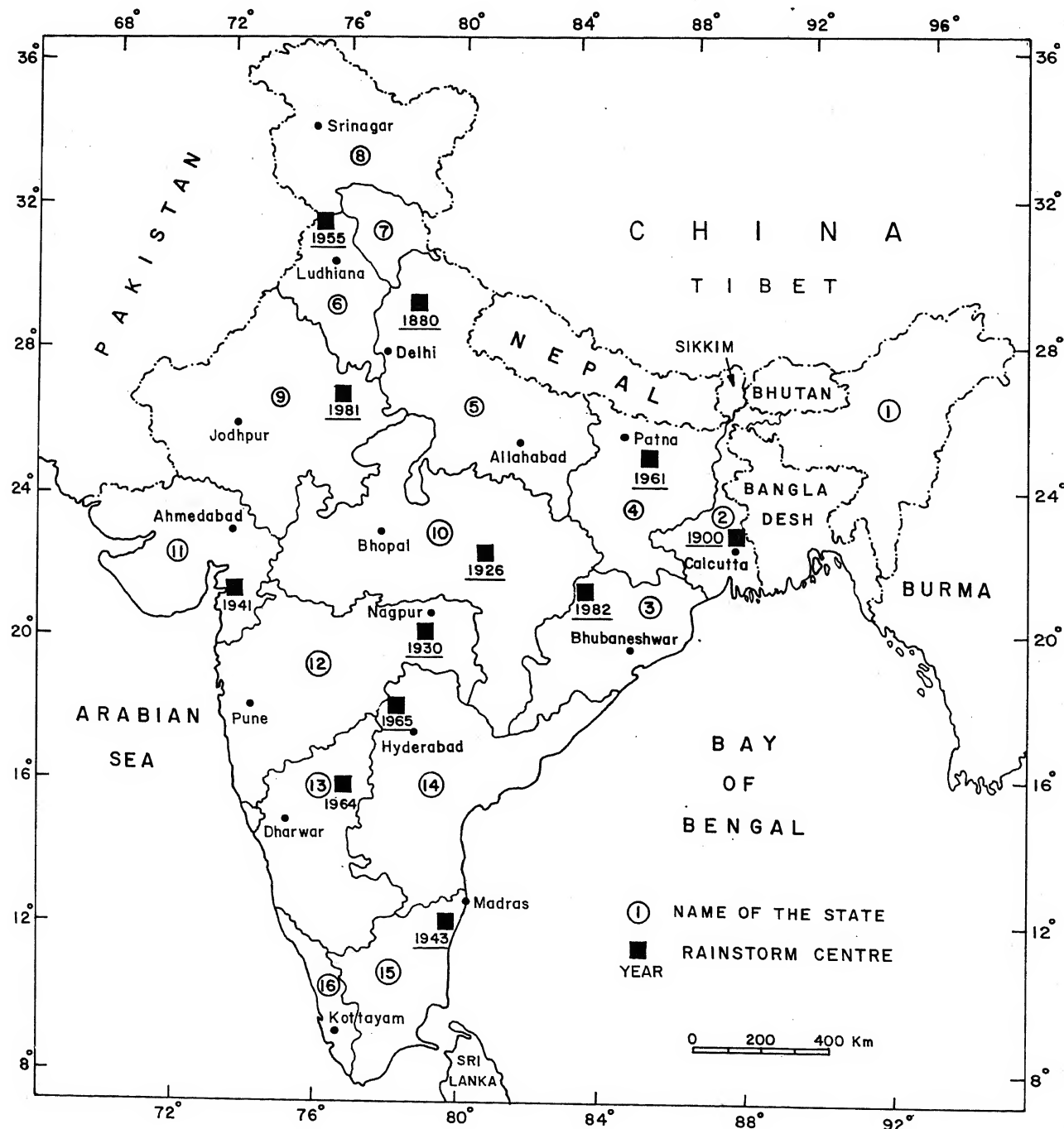


Figure 4. Locations of rainstorms centres.

highest rainfall values of one, two and three-day durations. It may be pointed out that there is an element of randomness in the magnitudes of the highest rainfall recorded even though observations are available for over 100 years. But when the data of all stations are examined together, some broad patterns emerge. The highest rainfalls for 1, 2 and 3-day durations were plotted separately on a large scale map of India and isolines drawn at suitable intervals. The resulting spatial patterns are shown in Figures 1 to 3.

Figure 1 shows the pattern of the highest 1-day rainfall across the Indian region. The isohyets of the highest 1-day rainfall range from less than 20 cm over large parts of the interior peninsula, the arid region of the west Rajasthan and northeast of Jammu and Kashmir to over 40 cm on and near the coastal strips including the Gujarat and Saurashtra coasts, the mountainous regions of the Western Ghats, the hills of Assam and the foothills of the Himalayas. Heavy rainfalls exceeding 30 cm in 1-day have also occurred over the central parts

of India lying between 19°N to 25°N and 70°E to 84°E. Some places on or near the coasts for example, Bombay, Harnai, Cuddalore, Wandiwash, Kakinada, Paradip, Gopalpur and Contai and some places in the hills such as Agumbe (Karnataka), Mount Abu (Rajasthan), Khandala (Maharashtra), Cherrapunji, Jowai and Mawsynram have recorded 60 to 100 cm rainfall in 1-day.

The spatial patterns of 2-day and 3-day highest rainfalls are shown in Figures 2 and 3 respectively. The highest recorded rainfall values of 1 to 3-day durations for some coastal as well as for high and low level stations are given in Table 2.

The highest rainfall values represent only small areas around the recording points. The table shows that Cherrapunji (mountainous area) recorded the highest rainfalls of 104 cm, 165 cm and 224 cm in 1, 2 and 3-day periods respectively. Dharampur (plain area) in south Gujarat recorded the highest rainfalls of 99 cm, 126 cm and 145 cm in 1, 2 and 3-day periods respectively. It was found that a cyclonic disturbance lasting for 6 days ravaged the area at that time and provided ideal conditions for spectacular falls.

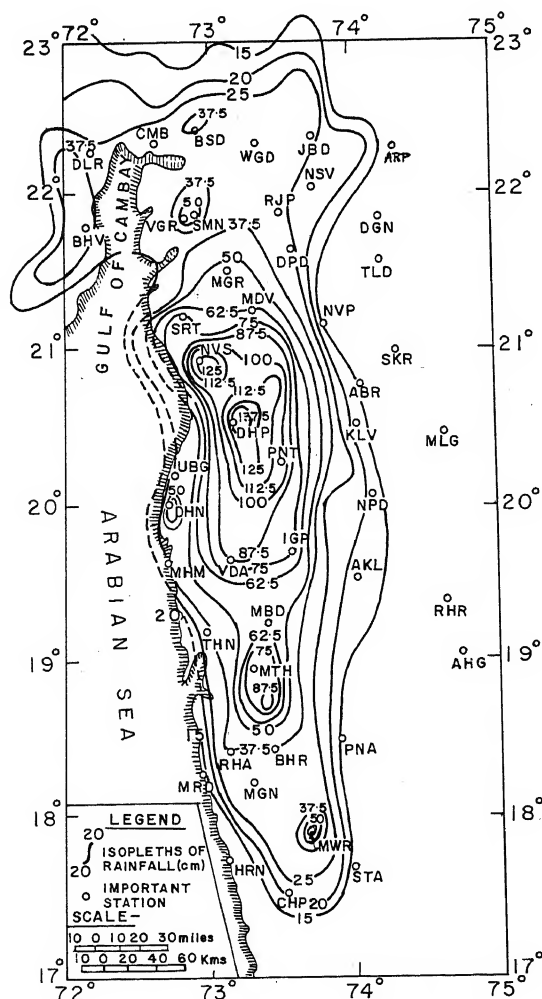


Figure 5. Rainstorm isohyetal pattern of 3 days (1-3 July 1941) over south Gujarat-North Konkan.

Table 4. Maximum 1-hour rainfall amounts

Station	Amount (cm)
Allahabad	7.5
Asansol	8.6
Amritsar	6.5
Aurangabad	6.1
Agartala	6.6
Ahmedabad	8.0
Baroda	6.7
Bombay	12.9
Bangalore	6.1
Bhopal	7.2
Cherrapunji	12.7
Calcutta	6.8
Dibrugarh	6.0
Gaya	7.0
Hyderabad	10.2
Hazaribagh	6.7
Hirakud	8.2
Jamshedpur	8.6
Jodhpur	5.1
Jaipur	5.7
Jabalpur	7.4
Jagdalpur	7.3
Kodaikanal	6.9
Lucknow	7.0
Madras	6.2
Mangalore	6.4
Mahabaleshwar	4.9
New Delhi	7.9
Nagpur	7.8
Pune	4.7
Trivandrum	7.1
Tiruchirapalli	7.8
Veraval	12.2
Vengurla	6.6
Visakhapatnam	6.3

Highest areal rainfalls

Heavy rainfalls are most important when the period extends to days and the area covered is extensive. Cyclonic disturbances (depressions/cyclonic storms) cause widespread and intense rainfall in areas over which they travel. During the SW monsoon season, the maximum number of cyclonic storms (mainly depressions) from over north Bay of Bengal which have usually a life period of 4 to 5 days. After formation they travel in a northwesterly direction across the country. Rainfall occurs in the region along and near the tracks of these moving storms. The total areal covered by the rainfall reaches about 400,000 km² and point rainfall ranges from 40 to 80 cm in 1-day. These monsoon depressions play a very critical role in the distribution of monsoon rains over the country.

The tracks of these disturbances in July and August are very significant. Their tracks in these two months are concentrated into a comparatively narrow band, indicating a certain amount of regularity in their movement during this period. During September, the spread of the storm tracks is noticeable and it increases further during the post-monsoon month of October. The larger spread of storm tracks indicates the possibility of heavy rainfall occurring not only in the northern river catchments, but also in the southern river catchments during September and October. Cyclones occur, some of them of severe intensity, during the premonsoon (March–May) and postmonsoon (October–December).

Recently, on the basis of rainfall data from the 15 highest storms that occurred in different parts of India, areal distribution maps of maximum 1-day, 2-day and 3-day rainfalls were prepared by IITM¹. Based on the

depth-area-duration (DAD) method, the largest average depth of rainfall that fell over various sizes of area during 1, 2 and 3-day durations are given in Table 3. For a 5000 km² area the maximum rainfall from these 12 storms varied from 22 to 65 cm for 1-day, from 27 to 97 cm for 2-day and from 38 to 117 cm for 3-day duration. The locations of the storms are shown in Figure 4.

The primary weather feature responsible for causing the above heavy rain spells were depressions/cyclonic storms from Bay of Bengal. The spatial pattern of rainfall for 3-day duration for the July 1941 storm is shown in Figure 5. The centre of the storm was located at the Dharampur station in the Gujarat region, which recorded 99 cm on the first day, 127 cm on the first and second day together, and 145 cm on the three days put together.

Highest hourly rainfalls

The highest 1-hour rainfall values for some stations are shown in Table 4. These figures represent intensities only over small areas around the recording points; for turbulence and exposure characteristics of the measuring gauge can vary even over a small distance of a few metres. Most of the very high 1-hour falls have occurred in the coastal and mountainous areas. The highest 1-hour fall, 12.9 cm occurred at Bombay.

1. Indian Institute of Tropical Meteorology, Severe Rainstorms of India Atlas, IITM Publ., 1994.

ACKNOWLEDGEMENT. We are grateful to Prof. R. N. Keshavmurthy, Director for constant encouragement.

Corrigendum

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Read 'Centre for Biochemical Technology' instead of 'Centre for Biotechnology' in lines 4 and 7.

Linking technology development with production – A methodology and case histories

S. C. Gupta

Significance and the process of linking technology development with production in the innovation chain are brought out. The individual and joint roles and responsibilities of the development and production agencies are identified. The methodology and attitude used for fruitful interaction between the development teams of VSSC and the different production agencies are illustrated by case histories of different items. A case of unsuccessful production is also presented to bring out the importance of market forces.

GLOBALIZATION of Indian economy has made pursuit of competitiveness by industry of the country an imperative. Generation and utilization of know-how for improved products and services contribute greatly to competitiveness. For this purpose, there has to be a strong team-work between the research and development teams – which generate the know-how, and the production teams – which utilize the know-how. Whether or not the two teams belong to the same corporate body, the team-work has to be meticulous. The data for India on expenditure on R&D by industry, utilization of know-how generated in India and import of know-how brings out unambiguously that there is little support for the generation of know-how in India and most of the need is met by import. India has a vast R&D infrastructure and substantial R&D manpower. India has also achieved impressive successes in developing and utilizing know-how in a number of important fields, such as space and agriculture. Thus, there is inherent capability. What is lacking is an effective harnessing of the inherent capability.

One of the most important functions of research and development is to generate know-how for transfer to industry for production of new and improved goods and services. Acceptance of the know-how by industry spurs R&D. However, acceptance depends on the techno-economic features of the know-how. The know-how has to be mature and comprehensive enough for smooth production. The respective responsibilities of the R&D and production teams during the transfer, production and marketing activities are to be identified clearly and discharged fully. Near certain prospects of attractive profits to the production agency strengthen acceptance. The R&D team can provide adequate information and

confidence on the first two features of the offered know-how. As the profit potential of the know-how also depends on factors such as market forces, beyond the control of laboratory and industry, the information generated by the R&D team has to be supplemented.

Lack of confidence in the dependability of the technical information provided by the R&D team is often a stumbling block. While critical analysis of the information by independent analysts and consultants may generate some confidence, naturally the clinching assurance comes from the earlier experience of working together successfully. An offer of systematic methodology of know-how transfer and team-work can also generate confidence, specially when past experience in know-how transfer from an R&D team is limited or non-existent. Since the production team is also intimately involved in the transfer of know-how, careful selection of the production team(s) is also critical for the success of the transfer process.

One of the motivations of technology development teams is to see the fruits of their labour being utilized by the needy users. Achieving higher performance and greater reliability at costs affordable to a large body of users is well worth the painstaking and innovative efforts to improve design, fabrication and testing processes. However, without ensuring production with specified quality and in needed quantities at required schedules and at competitive costs, the development process may not progress beyond the development laboratory and reach users. As a result, the developed product may eventually go to a museum rather than to the market place. Just as a development laboratory is the most likely place for innovation and technology development, a manufacturing facility is the most suitable place to do justice to the exacting demands of production. The development laboratory and manufacturing facility thus

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form indispensable links in the innovation chain. The user forms the third link in the chain. Vigorous interaction among the three links contributes greatly to the success of the innovation cycle. What needs to be done to bring about vigorous and fruitful interaction between a development laboratory and a manufacturing facility is the subject of this paper.

This paper draws on the experience of the Vikram Sarabhai Space Centre of the Indian Space Research Organization in technology development and transfer. Identified are the features of mature and comprehensive know-how, respective responsibilities of the R&D and production teams and selection process of production team(s). A few case histories covering both successful and unsuccessful production are also presented to illustrate and clarify the issues.

Features of know-how

To enable taking up production, the production team requires the following essential details:

(i) A mature design, based on which the product has been made and has undergone successfully independent rigorous performance evaluation and service environment-endurance tests. Product should have been made in enough quantities to establish repeatability of performance within the identified tolerance band. While the design methodology does not have to be revealed, full description of materials of construction, component values and permissible tolerances, etc. is essential. A comprehensive guideline is given in ref. 1. (ii) Data on performance evaluation and environmental endurance test results. (iii) Drawings and explanatory notes using which the product can be made by a team skilled in the relevant fields, without the direct participation of the development team. Those components or processes are to be highlighted, successful realization of which requires unusually high skills of craftsmanship, meticulous quality checks and/or specialized facilities. (iv) Information on the required raw materials and components and their quality standards and sources of supply. (v) Information on quality control processes to be adopted during the various phases of production. (vi) Information on equipments for production, quality control, performance evaluation and environmental tests. (vii) Information on maintainability, servicing, essential spares, and recalibration procedures, wherever applicable. (viii) Data on consumption of energy and raw materials in the production of the product, wherever applicable. (ix) Information on effluents and their likely impact on environment and ecology with or without effluent treatment. Process and cost of effluent treatment, if any. (x) Information on hazards of any kind, if any, in the production process and effluent treatment. (xi) Informa-

tion on assured demand for the product in terms of quantity, quality and pricing over a reasonable period of time or till the product is likely to become obsolete. (xii) Information on likely time by which the product will become obsolete.

The development team should be able to generate adequate data on the first five items listed above. Data on the rest may have to be generated jointly by the development and the production teams and the users, even seeking the assistance of other specialist teams. The need to generate adequate data on the items listed above, in order not to have surprises in the technical aspects during the production phase cannot be over-emphasized.

Pooling of information with producers and users

Just as the production team requires certain essential information from the development teams and the users, the development team requires the following inputs from the users and the production team.

(i) Survey of the existing comparable products, their performance characteristics, quality standards, pricing and improvements desired by users. (ii) Survey of trends which may influence the onset of obsolescence of the product. (iii) Information, if any, from value engineering point of view, which may help in cost reduction without affecting performance. This could include use of alternate materials, processes, etc. (iv) Data on facilities accessible to the production team for production, inspection, performance evaluation and environment tests, so that the minimization of need for new facilities can be an objective of the development team. (v) Data on availability, quality standard, and prices of raw materials and components.

Management support

Any activity involving teams, organizations, funds and schedule needs management support. Individual and common responsibilities of the participating persons and organizations are to be spelled out in detail. Periodic reviews are to be carried out at the different managerial levels concerning technical, financial and schedule-related matters. For the sake of brevity, these are not elaborated here. However, the most critical factor concerning the attitude of all those who are responsible for implementing and reviewing the activities needs to be discussed here. A memorandum of understanding (MOU) between the participating organizations is generally entered into covering the various aspects of technical, financial and schedule-related matters. The MOU is a charter for

actions under foreseeable conditions. However, when unforeseen situations arise, the MOU has always to be supplemented by the will and determination to succeed. This may call for amendments, to the MOU placing varying additional burdens on the participants. Further, the participating teams should be made to feel that the product is the outcome of their joint efforts rather than that of the development team alone. The knowledge and experience base of both the development and the production teams needs to be used in bringing out the product. Admittedly, the development team may have an edge over the production team in areas of design, technology, craftsmanship skills and performance evaluation. Likewise, the production team may have an edge over the development team in areas of production processes and equipments, sources of materials and components, costs involved in achieving quality standards, economizing on materials and energy consumption.

Technology upgradation

It is clear that passing on a product for production entails design and technology freeze. Does it mean that in this scheme of things there is no scope for improvement of design, and technology upgradation and that the innovation process is given up once the user's requirements are apparently met? Fortunately, the innovation process is continued relentlessly. However, the utilization of innovation is carried out in a qualitatively different manner. In this context, it is useful to recognize that innovation process results in a monotonic rise with time in the values of parameters representing performance, quality and productivity. The rate of rise may vary depending on achievement of major or minor breakthroughs during the innovation process. The design and technology freeze, implying the keeping of the product parameters at a fixed level, results in growth of gaps between the realizable and the frozen values of the product parameters. Upgradation of the design and technology cannot be carried out as and when the development team accomplishes them, but is carried out subsequently when the cost of implementing the upgradation is justified by the benefits of the improved product. Thus the upgradation adopted in production is like climbing up a step. This process may be repeated over a period. From the above, it is recognizable that while the product parameters undergo a continuous ramp-like growth due to innovation, they are adopted in a staircase-like manner in the production process. Over a long term the parametric levels in the innovation and production processes are nearly the same, however, at any point in time the parameters adopted for production may in general be inferior to those achieved in innovation.

Periodic quality audit

Once the production process is stabilized and the sources and quality parameters of inputs, raw materials and bought out parts are standardized, the product quality is well assured. However, small and slow changes may occur in the production process and the parameters of inputs due to a number of reasons, such as, wear of production equipment, drift in calibration of crucial measuring instruments, turn-over of critical personnel and change of suppliers of inputs. Such imperceptible changes can seriously affect the quality and yield of the product. A periodic quality audit addressing the above-mentioned parameters is therefore essential in order to maintain trouble-free production.

Some unusual aspects

The above mentioned list of items on which data has to be generated for undertaking production is fairly obvious. However, such issues as dispersal of the production process to more than one institution in order to utilize facilities wherever they may exist and thus minimize setting up of new facilities may not surface usually. In the production of advanced technology products, generally requiring large capital outlays, this issue is of major concern. If significantly expensive new facilities are essential, additional capital costs and facilities-build-up time will greatly influence the production costs and time to take the product to users. On the other hand, if the entire production process is not under one roof, smooth flow of production would require well-conceived coordination between the participating facilities. This can add to the uncertainties regarding the uninterrupted availability of the product to the users. A trade-off study to determine how much should be under one roof and rest can be dispersed may be necessary. The trade-off study may also suggest progressive variation of the dispersal management.

Indirect benefits to a production agency of undertaking production of advanced technology products with stringent quality assurance norms are not commonly recognized, although a little reflection should make the benefits obvious. The following brings out the benefits. Some examples of the quality assurance requirements peculiar to advanced technology product are:

- (i) The production process is divided into a suitable number of stages and in-process stage inspection is carried out preferably by the process operators themselves. This is in contrast to the usual practice of inspection by an inspection team only at the end of the production process. Under the modified procedure, the production process is allowed to continue without interruption only if the results of stage inspections are

satisfactory. Failure in stage inspection, however, calls for rework or rejection, thus avoiding infructuous processing effort and possible waste of material. This also increases the involvement and dedication of the process operators to the achievement of quality; (ii) On-line recording and analysis of production processes and inspection activities are given in ref. 2. These bring out (a) whether the production process wandered outside the specified tolerances at any stage, (b) what rework was carried out, if any, and (c) whether any performance parameters of the product are outside the specified limit.

A review of the above quality assurance data permits confident decision on the suitability of the product for the intended service by exacting users. This data also provides traceability to be able to correlate failure of the product during service with lapses, if any, during the design and production processes. Beneficial influence of such quality assurance practices on the production yield and product quality enhances the image of the production agency.

Successful production of a newly developed product has another significant, though intangible, benefit. It is the growth of mutual confidence between the development and production teams to be able to undertake challenging development and production tasks. Likewise, the user community begins to rely on such products and expects more of such successful collaborations.

Selection of production agency

The production agency plays the critical role of implementing the know-how to produce and market the new goods and services with the human and infrastructural resources accessible to them. Easy access to the needed skilled manpower and facilities for production, quality control and test and evaluation are the minimum prerequisites of a suitable production agency. If the new products and services are in line with the existing products and services of the prospective production agency, the task of successful production and marketing becomes simpler. If the production agency also has the motivation and internal capability of further value addition to the know-how, it becomes an excellent additional qualification for the selection of the production agency. An open meeting with the prospective production agencies, in which the various features of the know-how are presented by the R&D team and the capabilities of the production teams are discussed individually, can prove to be a speedy step in the selection process. Exchange of visits to the R&D laboratories and production facilities is another useful step in the selection process. Ready agreement on sharing of responsibilities during the technology transfer, implementation and marketing activities creates proper conditions for successful production.

Case histories

The concepts of linking technology development with production have been used extensively in the Indian Space Research Organization (ISRO) in general and the Vikram Sarabhai Space Centre (VSSC), in particular. Four case histories are cited here for illustration. These are concerning production of: (i) Miniature rate gyroscope; (ii) Electronics assemblies; (iii) Carbon cloth and (iv) Telemetry systems representing different fields. There are other numerous examples linking development with production in the fields of alloys, polymers, liquid fuels, large variety of mechanical components and assemblies and software which are not discussed in this paper for the sake of brevity and as the selected case histories discussed in some detail illustrate the concepts sufficiently.

Miniature rate gyroscope

VSSC had developed a highly sophisticated electro-mechanical instrument for measuring the speed of rotation of solid bodies, undergoing complex motion, and producing a proportional electrical signal with stringently specified precision and accuracy. The instrument, operating on the principle of conservation of angular momentum and interaction of gyroscopic torques, is known as a rate gyroscope. The main sub-assemblies comprising the gyroscope are a spin motor usually running at 24000 rpm, a torsion bar, a microsyn, a flotation system and a pivot-jewel assembly. The sub-assemblies have a total of 16 high precision fabricated parts and a number of bought-out parts. The physical size of the instrument puts it in the miniature category.

During the development phase, the VSSC team evolved the geometry and the material for the parts, the processes for machining, fabrication and heat treatment, the assembly procedures and performance checks on the sub-assemblies, the process tools required during the production and assembly and procedure for assembly and performance evaluation. The development team also evolved the detailed quality assurance procedures concerning raw materials, bought-out parts and production and assembly process. After achieving the required performance of the gyroscope consistently over a number of units, the design, fabrication, assembly, performance evaluation and quality assurance information, as listed earlier, was documented as a Technology Transfer Document. Then, the search for a suitable production agency was made. A facility which was producing instruments based on comparable technologies under licence from a number of foreign manufacturers was located. After discussing with the production facility and agreeing on the terms of the order and the minimum significant size of the order, a two-phase production order was placed

with provision for total buy-back. In the first phase, only the full sets of the 16 fabricated parts were to be produced. In the second phase, which was to commence only after completing successfully the first phase, the complete instrument was to be produced. In order to reduce the uncertainties on schedule, quality and cost all special raw materials and bought-out parts were supplied by VSSC.

When the production agency commenced the fabrication of parts, difficulties were experienced in realizing the stringent geometrical tolerances on some of the parts. Difficulties were also experienced in achieving quality standards in soldering electrical connections, miniature coil winding and inspection of precision engineered parts. Some difficulties also arose because the production agency was following the method of doing inspection at the completion of the fabrication and machine shop work. Stage inspection was not in vogue. Because of this practice, the shop floor technicians were not issued the precision gauges needed for stage inspection. As a result, the non-conformance with the specified geometrical tolerances was detected only at the completion of the fabrication and machine shop work. Consequently, the yield was poor for even those parts which the production agency could manage to produce in the early trials. In the management level review, these difficulties were brought up and the following remedial action were identified for implementation:

(i) Training-cum-demonstration of fabrication, machining, soldering, miniature coil winding and stage inspection of the difficult-to-produce parts to the technicians, inspectors and supervisors of the production facility at the development laboratory. If necessary, repeat the demonstration of producibility at the production agency using facilities available there by the development laboratory technicians. (ii) Introduction of stage inspection at the production agency, entailing increase in the scope of responsibility of the production technicians and for this purpose equipping them with the required precision gauges.

During the subsequent management level reviews, while significant improvement in the yield of most of the parts was observed, as a result of the above mentioned measures, a few parts continued to elude satisfactory production. In order to solve this problem and to improve the yield further, the production agency decided to set up a separate Cell in the facility to fabricate and machine the gyro parts and to equip the Cell with a few special machine tools recommended by the VSSC development team. On registering satisfactory progress on the first phase, the second phase was taken up concurrently with the first phase, as a modification of the earlier plan.

The gyroscopes coming from this production arrangement have been available to the entire launch vehicle

programme of ISRO from SLV-3, ASLV to PSLV and have never been cause of the failure of the launch vehicle missions. All through the working together, the development and production teams maintained the spirit of searching for solutions rather than alibis for failures and of strict adherence to systematic quality assurance procedures.

Electronics assemblies

The space launch vehicles and satellites have on-board a large number of special electronics assemblies for performing the functions of measurement, signal processing, data acquisition, telemetry, trajectography, telecommand, attitude control, electric power conditioning, flight sequencing, etc. These assemblies span the frequency spectrum from d.c. to several Gigahertz and are of both analog and digital type. The electronics components used in them are of high reliability standard and the workmanship standards on components preparation, mounting, soldering, harness preparation are of equally high reliability standard, so that the assembly can successfully withstand the hostile environment during launch and in-orbit operation in space. The quality and workmanship standards are given in refs 3-7. For the assemblies to deliver performance in a narrow tolerance band in terms of gain, frequency and voltage stability, over a wide temperature environment, the component values need to be in correspondingly specified narrow bands. Tuning, performance checking and quality inspection at various stages of the production are in general indispensable, in order to get satisfactory yield from the production.

After the satisfactory completion of the design and development process at VSSC, production documents were prepared incorporating the information listed earlier in this paper. A number of suitable production agencies were identified and contracted with the production of the electronics assemblies used in the ASLV, PSLV and the Rohini Sounding Rockets. Although the technicians, inspectors and supervisors at the production agencies were reasonably well skilled, it was found necessary to give them additional training in the techniques of making high reliability electrical connections, spotting sub-standard connections and repairing the sub-standard connections while maintaining the quality standards. It was also found necessary to introduce stage inspection during the production of printed circuit boards and the final assembly operations.

During the periodic management level reviews, the spirit of problem solving in order to achieve the goals was maintained by the development and production teams.

Currently, about 38 varieties of electronics assemblies, ranging from the simple signal conditioning units to complex guidance and control computers, are being produced in industry. The electronics assemblies so

produced have given faultless performance in the ASLV, PSLV and RSR flights. A few of these electronics sub-assemblies are also used completely satisfactorily in the IRS-1A, 1B, 1C and INSAT-2A, 2B, 2C satellites providing in-orbit services.

Carbon cloth

The nozzle of the solid propellant rocket motors is lined with layers of phenolic-resin impregnated carbon cloth. The basic function of these layers is to maintain the internal dimensions and the strength of the metallic structures of the nozzles under supersonic gaseous flow at temperature higher than 2000°C and the flow lasting up to two minutes. The carbon cloth is produced by carbonizing a special grade of rayon cloth, having a particular type of weave pattern. The carbonization is a multi-stage process and is to be carried out in furnaces with special liquid medium and gaseous environment and at different temperatures going up to about 1000°C. The carbon cloth is about 1 m wide and in continuous lengths of not less than 50 m. Specifications of the cloth in respect of carbon and sodium contents, breakstrength in warp and fill directions, thickness and mass per unit area have stringent tolerances. There are only a handful of qualified producers in the whole world of this grade of the carbon cloth and the technology of production is a closely guarded commercial and strategic secret.

VSSC had developed *de novo* the carbon cloth production process in 40 mm width and 500 mm length size and prepared a detailed document giving the desired rayon cloth characteristics, the carbonization process and quality control procedures. The production agency, identified through a competitive selection process, was to scale up the carbonization process and adopt a batch or continuous process to produce the carbon cloth with the specified characteristics. The selected production agency turned out to be a brand new venture, entailing the *de novo* design and procurement of the process equipments and design and construction of the plant buildings. VSSC organized for them a technology demonstration programme in order to (i) confirm the reproducibility of the lab scale carbon cloth processing technology, (ii) familiarize with the processing testing and evaluation techniques, and (iii) enable them to have an independent verification of the technologies under transfer. During the selection of the process equipments and the scaling up exercises the development team provided specialist advice. The initial technical difficulties faced in commissioning the process equipments, evolving the scaled up process, analysis of cloth samples, improving the yield were painstakingly reviewed and overcome. The production agency also came up with some useful innovative ideas. The carbon cloth is now

being produced regularly, fully conforming to the specified quality standards and used in the solid propellant rocket motors of VSSC.

Telemetry systems

ISRO had developed a complete set of telemetry system for monitoring the inflight performance of sounding rockets and satellite launch vehicles and the in-orbit health parameters of satellites. Both the on-board and ground segments and both analog and digital versions were developed and used extensively in over hundred missions of Rohini Sounding Rockets, SLV-3 vehicle, ARYABHATA and BHASKARA satellites. The performance of the telemetry system was invariably spot-less and helped ISRO extract valuable information from these missions. On the basis of this performance record a specially configured telemetry system was designed, built and supplied to Hindustan Aeronautics Ltd for use in their new aircraft developments. Encouraged by these achievements, ISRO tried to interest Indian industry to undertake production of the telemetry system for application to the massive oil and gas pipeline projects in the country. A workshop on Application of Telemetry in Industry was held followed by an ISRO-industry meet on the theme. In spite of the proper credentials of ISRO and strenuous efforts to familiarize industry with the capabilities and the potential market for the telemetry system, imported know-how won the pipeline application. Apprehending similar preference in other applications, industry did not select the know-how from ISRO. Thus, this constitutes a case of unsuccessful production for non-space applications.

1. 'Design Review Requirements for ISRO Projects', ISRO-PAS-200, Jan. 1982.
2. 'Failure Reporting, Analysis and Corrective Action Procedures', ISRO-PAS-201, May 1982.
3. 'Workmanship Standards for Fabrication of Electronic Packages', ISRO-PAX-300, December 1983.
4. 'Design Requirements for Printed Circuit Board Layout and Artwork', ISRO-PAX-301, September 1983.
5. 'Test Specification for Printed Circuit Boards', ISRO-PAX-302, November 1985.
6. 'Design Requirement for Multilayer Printed Circuit Board Layout and Art Work', ISRO-PAX-303 (1), December 1987.
7. 'Screening Requirements for Electronics Parts', ISRO-PAS-204, December 1984.

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Viral cell recognition and entry

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Rhinovirus infection is initiated by the recognition of a specific cell surface receptor. The major group of rhinovirus serotypes attach to intercellular adhesion molecule-1 (ICAM-1). The attachment process initiates a series of conformational changes resulting in the loss of genomic RNA from the virion. X-ray crystallography and sequence comparison suggested that a deep crevice or canyon is the site on the virus recognized by the cellular receptor molecule. This has now been verified by electron microscopy of human rhinovirus 14 (HRV14) and HRV16 complexed with a soluble component of ICAM-1.

A hydrophobic pocket underneath the canyon is the site of binding of various hydrophobic drug compounds which can inhibit attachment and uncoating. This pocket is also associated with an unidentified, possibly cellular in origin, 'pocket factor'. The pocket factor binding site overlaps the binding site of the receptor. It is suggested that competition between the pocket factor and receptor regulates the conformational changes required for the initiation of the entry of the genomic RNA into the cell.

Viral receptors

Unlike plant viruses, most animal, insect and bacterial viruses attach to specific cellular receptors that, in part, determine host range and tissue tropism. Viruses have adapted themselves to utilize a wide variety of cell-surface molecules as their receptors, including proteins, carbohydrates and glycolipids. Some viruses recognize very specific molecules (e.g. a large group of rhinoviruses recognize intercellular adhesion molecule-1 (ICAM-1)), while other viruses recognize widely distributed chemical groups (e.g. influenza viruses recognize sialic acid moieties). The tissue distribution of the receptor will in part determine the tropism of the virus and, hence, the symptoms of the infection. Similarly, species differences between receptor molecules can limit host range. For instance, only humans and apes have been shown to be susceptible to rhinovirus infections, a property correlated to the inability of human rhinoviruses to bind to the receptor ICAM-1 molecule in other species.

Although there are extensive similarities of sequence, structure and physical properties among picornaviruses which show that these viruses have evolved from a common ancestor¹⁻³, nevertheless they recognize a variety of receptors (Table 1). Possibly the primordial virus had

the ability to weakly bind to a large number of different molecules. With time, different viruses evolved which became progressively more efficient and specialized towards recognizing one particular molecule as a way of infecting specific cells. Indeed, the grouping of viruses might suggest such a scenario. Thus, all polioviruses appear to recognize the same receptor and most coxsackie A viruses recognize their own receptor, while coxsackie B viruses recognize yet another receptor. Therefore, it is surprising that rhinovirus serotypes can be divided into three groups which recognize different receptors^{4,5}. Furthermore, the receptor for the major group of rhinoviruses, ICAM-1, belongs to the immunoglobulin superfamily^{6,7}, whereas the receptor for the minor group has been reported to be the low density lipoprotein (LDL) receptor⁸.

Receptor binding is only the first, albeit essential, step in the infection process. The virus, or the virus genome alone, then has to enter the cell, a process which requires translocation of the viral genome or a sub-viral particle across the membrane into the cytoplasm, and, in some cases, into the nucleus. Since delivery of the viral genome into the cell involves major rearrangements of the capsid structure, entry must be a tightly regulated process which is triggered by the cell. The mechanism of entry can be, in the case of enveloped viruses, by fusion of the viral envelope with the limiting cellular membrane (Figure 1). This process has been well characterized in several viruses (Semliki Forest virus (SFV), influenza virus, Sendai virus) where fusion is induced by specific viral envelope proteins, activated by conformational changes induced by the low pH environment of endosomes. The mechanism by which protein-encapsidated viruses like picornaviruses³ enter the cytoplasm has not been well elucidated, but must differ significantly in detail from the membrane-fusion strategy demonstrated by enveloped viruses in that RNA must be translocated through the membrane.

Rhinovirus structure and the canyon hypothesis

The genus *Rhinovirus* is composed of a group of over 100 serologically distinct viruses, which are a major cause of the common cold in humans³. These viruses belong to the picornavirus family, which also contains the genera *Enterovirus*, *Aphthovirus*, *Cardiovirus* and

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Table 1. Receptors families for picornaviruses based on virus competition for cell receptors

Virus	Receptor molecule	Receptor family	Reference
Human rhinovirus major group: 78 serotypes, including 3, 5, 9, 12, 14, 15, 22, 32, 36, 39, 41, 51, 58, 59, 60, 66, 67, 89	ICAM-1	Ig (5 Ig domains)	Abraham and Colonno ⁴ , Greve <i>et al.</i> ⁶ , Staunton <i>et al.</i> ⁷
Human rhinovirus minor group: 11 serotypes, including 1A, 2, 44, 49	Low-density lipoprotein (LDL) receptor	LDLR	Abraham and Colonno ⁴ , Hofer <i>et al.</i> ⁸
Polioviruses	Poliovirus receptor (PVR)	Ig (3 Ig domains)	Mendelsohn <i>et al.</i> ³⁵
Coxsackievirus A13, 18, 21	ICAM-1	Ig (5 Ig domains)	Colonno <i>et al.</i> ⁸⁴ , Roivainen <i>et al.</i> ⁸⁵
Coxsackievirus A2, 5, 13, 15, 18	?	?	Colonno <i>et al.</i> ⁸⁴ , Roivainen <i>et al.</i> ⁸⁵ , Schultz and Crowell ⁸⁶
Echovirus 1	VLA-2	Integrin	Bergelson <i>et al.</i> ⁸⁷
Echovirus 6	?	?	Crowell ⁸⁸
Foot-and-mouth disease viruses, types A ₁₂ 119, O _{1B} , C _{3Res} :SAT ₁₋₃	RGD integrin	Integrin	Sekiguchi <i>et al.</i> ⁸⁹ , Mason <i>et al.</i> ⁹⁰
Mengo virus	?	Glycophorin (?)	Burness ⁹¹ , Burness and Pardoe ⁹²

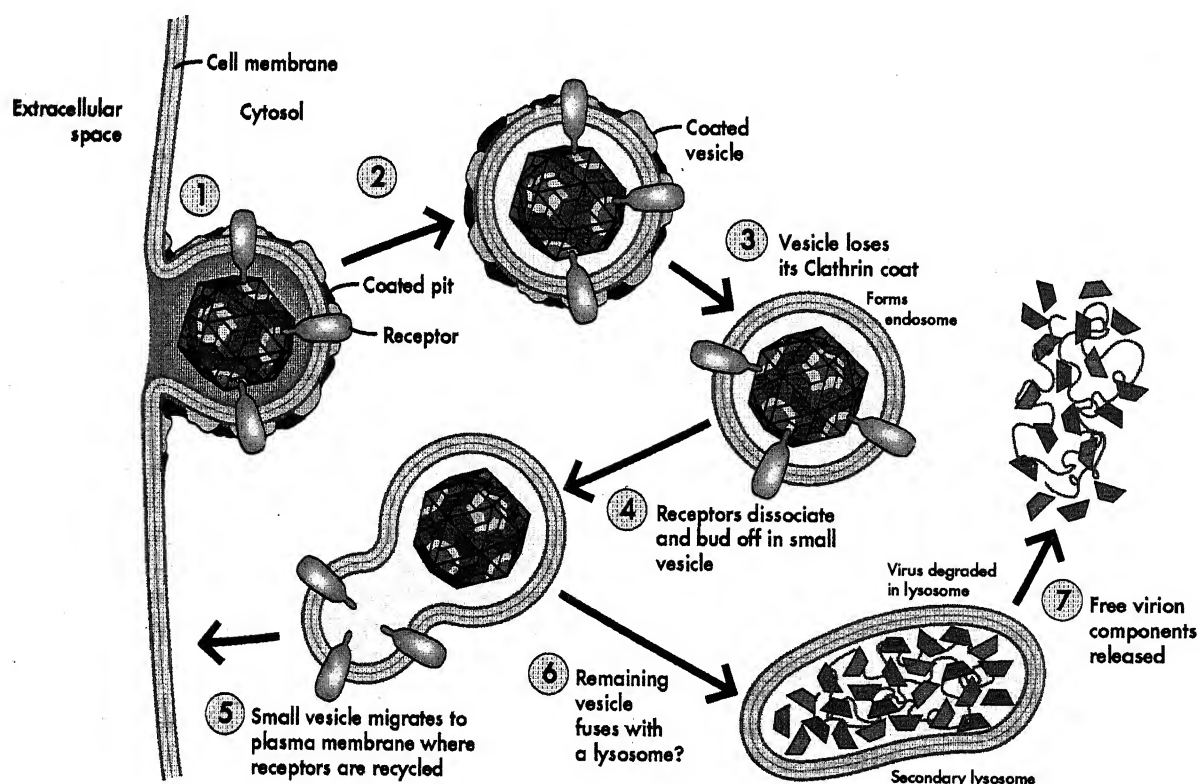


Figure 1. One possible endocytotic process (adapted from Rawn¹⁰³). Note, however, that in most cases it is not known when and where the receptor and virus part company, whether it is necessary for the virus to be bound to the receptor during uncoating and what is the mechanism by which RNA translocates the membrane.

Hepatitis A virus. The picornaviruses are small, icosahedral, nonenveloped, single-stranded RNA viruses. X-ray crystal structures have been determined for at least one member in each picornavirus genus except for hepatitis A viruses^{1,9-13}. Polioviruses (genus *Enterovirus*) are structurally the most similar to rhinoviruses. Unlike the enteroviruses, rhinoviruses are unstable below pH 6. The infectious virion has a molecular weight of about 8.5×10^6 daltons and an external diameter of around 300 Å.

Each of the 60 icosahedral protomers in picornaviruses contains four viral polypeptides, VP1-VP4. VP1, VP2 and VP3 reside on the exterior of the virus and make up its protein shell (Figure 2). These three peptides, each having a molecular weight of roughly 35 kDa, contain a common eight-stranded, antiparallel, β -barrel motif¹ (Figure 3, Table 2). Their amino termini intertwine to form a network on the interior of the protein shell. Five VP3 amino termini form a five-stranded helical β -cylinder on the virion's interior about each icosahedral five-fold axis. This β -cylinder stabilizes the pentamer and is thought to be important for its assembly^{9,14}.

VP4 is smaller than the other viral polypeptides and resides inside the virion's protein shell. VP4 is lost from the capsid as a result of virus uncoating, although the specific role of VP4 in uncoating or entry has not been elucidated. A mutant of human rhinovirus serotype 14 (HRV14) defective in VP4-VP2 cleavage¹⁵ is able to bind to receptor and undergo cell-induced conformational transitions but is unable to initiate a new round of replication, suggesting that cleavage of VP0 into VP2

and VP4 (cf. refs 10, 14) is an essential prerequisite for successful cell infection. The amino terminus of VP4 is myristylated, which may promote its association with lipid membranes during viral assembly or uncoating¹⁶. In poliovirus, the myristylate moiety lies inside the virion coat close to the β -cylinder. The first 25 to 28 amino-terminal residues of VP4 are mostly disordered in rhinovirus structures, but density consistent with myristylate is seen internally near the center of the pentamer in rhinoviruses 14, 1A and 16 (refs 13, 17, 18).

Each of the three larger capsid proteins has various insertions between the β -strands of the basic folding motif. Many of these insertions decorate the viral exterior and form 'puffs' and loops which are hypervariable and have been shown to be the binding site of neutralizing antibodies^{1,19,20}. The surfaces of rhinoviruses (and polioviruses) contain a series of remarkably deep crevices or 'canyons' (Figure 2), unlike anything observed in plant virus structures. The canyon is formed roughly at the junction of VP1 (forming the 'north' rim) with VP2 and VP3 (forming the 'south' rim). The GH loop in VP1 (often referred to as the 'FMDV loop' because of its immunodominance in the homologous foot-and-mouth disease virus (FMDV) structure) forms much of the floor of the canyon. Together with the carboxy termini of VP1 and VP3, the GH loop of VP1 also participates in the formation of the 'south' rim of the canyon.

It was hypothesized¹ that the canyon (one around each five-fold vertex; Figure 2) in HRV was the site of receptor attachment, largely inaccessible to the broad antigen-binding region seen on antibodies. Thus, residues

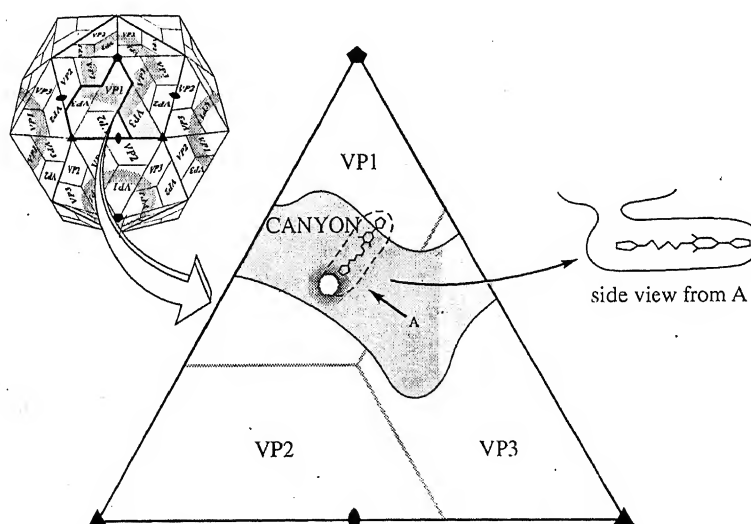


Figure 2. Top left: Diagrammatic view of picornavirus showing VP1, VP2 and VP3 and the deep cleft or 'canyon' running around each five-fold vertex. The 6S protomeric assembly unit (which differs from the geometric definition of the asymmetric unit) is shown in heavy outline on the icosahedron. Center: Enlargement of one icosahedral asymmetric unit showing the outline of the canyon and the entrance to the VP4 pocket. The terms 'north' (top) and 'south' rims of the canyon refer to this standard orientation. [Reprinted with permission from Oliveira *et al.*¹⁸. Copyright by *Current Biology*.]

in the lining of the canyon, which should be resistant to accepting mutations that might inhibit receptor attachment, would avoid presenting an unchanging target to neutralizing antibodies. Indeed, the neutralizing immunogenic sites that had been mapped by escape mutations were not in the canyon, but on the most exposed and variable parts of the virion both in HRV^{1,19,20} and in poliovirus^{9,21}. The 'canyon hypothesis' suggests that one strategy for viruses to escape the host's immune surveillance is to protect the receptor attachment site in a surface depression (Figure 4). Similar depressions related to host cell attachment have also been found on the surface of the haemagglutinin spike of influenza virus^{22,23}, tick-borne encephalitis virus (Harrison *et al.*, private communication) and may be the case for human immunodeficiency virus²⁴.

Binding of ICAM-1, the major group rhinovirus receptor, to virus surface

There are at least 78 serotypes²⁵ that bind to ICAM-1, the major group rhinovirus receptor^{6,7}. The ICAM-1 molecule has five immunoglobulin-like domains (D1–D5,

numbered sequentially from the amino end), a transmembrane portion and a small cytoplasmic domain^{26,27}. Domains D2, D3 and D4 are glycosylated (Figure 5). Unlike immunoglobulins, ICAM-1 appears to be monomeric⁷. Mutational analysis of ICAM-1 has shown that domain D1 contains the primary binding site for rhinoviruses as well as the binding site for its natural ligand, lymphocyte function-associated antigen-1 (LFA-1)^{27–30}. Other surface antigens within the immunoglobulin superfamily that are used by viruses as receptors include CD4 for human immunodeficiency virus type 1 (refs 31–34), the poliovirus receptor³⁵ and the mouse coronavirus receptor³⁶. In ICAM-1 in the poliovirus receptor^{37,38} and in CD4³⁹, the primary receptor virus binding site is domain D1. The structures of the two amino-terminal domains of CD4 have been determined to atomic resolution^{40–42}. Truncated proteins corresponding to the two amino-terminal domains of ICAM-1 (D1D2 consisting of 185 amino acid) as well as the intact extracellular portion of ICAM-1 (D1–D5 consisting of 453 amino acids) have been expressed in CHO cells⁴³. The desialated form of D1D2 has been crystallized⁴⁴.

The structure of the complex of D1D2 with HRV16 (ref. 45) and with HRV14 (P. R. Kolatkar, N. H. Olson,

Table 2. The common β -barrel fold

Virus ^a	Kingdom	Symmetry of capsid	Genome	Comments ^b	First reference
Plant					
TMV	Plant	Helical	RNA		
TBSV	Plant	$T=3$	RNA	1	Harrison <i>et al.</i> ⁹³
SBMV	Plant	$T=3$	ss + RNA	1	Abad-Zapatero <i>et al.</i> ⁹⁴
STNV	Plant	$T=1$	ss + RNA	1	Liljas <i>et al.</i> ⁹⁵
CPMV	Plant	Pseudo $T=3$	ss + RNA	1	Stauffer <i>et al.</i> ⁹⁶
BPMV	Plant	Pseudo $T=3$	ss + RNA	1, 2	Chen <i>et al.</i> ⁹⁷
STMV	Plant	$T=1$	ss + RNA	1, 2	Larson <i>et al.</i> ⁹⁸
Insect					
BBV	Insects	$T=3$	ss + RNA	1	Hosur <i>et al.</i> ⁹⁹
FHV	Insects	$T=3$	ss + RNA	1, 2	Fisher <i>et al.</i> ¹⁰⁰
Bacterial					
ϕ X174	<i>E. coli</i>	$T=1$	ss + DNA	3, 4	McKenna <i>et al.</i> ¹⁰¹
Animal					
Influenza	Human	Globular head haemagglutinin spike	ss + RNA	1	Wilson <i>et al.</i> ²²
Adeno	Human	Capsid hexon		3	Roberts <i>et al.</i> ¹⁰²
HRV 14, 1A, 16	Human	Pseudo $T=3$	RNA	1	Rossmann <i>et al.</i> ¹ , Kim <i>et al.</i> ¹³ , Oliveira <i>et al.</i> ¹⁸
Coxsackievirus B3	Human	Pseudo $T=3$	RNA	1	Muckelbauer <i>et al.</i> , in preparation
Polio 1, 2, 3	Human	Pseudo $T=3$	RNA	1	Hogle <i>et al.</i> ⁹
Cardio	Mice	Pseudo $T=3$	RNA	1	Luo <i>et al.</i> ¹⁰
FMDV	Cattle	Pseudo $T=3$	RNA	1	Acharya <i>et al.</i> ¹¹
Parvo	Dogs and cats	$T=1$	ss + DNA	3, 4	Tsao <i>et al.</i> ⁶⁵

^aBBV, Black beetle virus; BPMV, beanpod mottle virus; CPMV, cowpea mosaic virus; FHV, flock house virus; SBMV, southern bean mosaic virus; STMV, Satellite tobacco mosaic virus; STNV, Satellite tobacco necrosis virus; TBSV, tomato bushy stunt virus; TMV, tobacco mosaic virus.

^b1 – There are mostly small insertions between β -strands.

2 – There is a significant amount of ordered RNA.

3 – There are very large insertions between β -strands.

4 – There is some ordered ss + DNA.

C. Music, J. M. Greve, T. S. Baker and M. G. Rossmann, unpublished results) and of D1D5 with HRV16 (Kolatkhar *et al.*, unpublished results) has been determined using cryoelectron microscopy and image reconstruction procedures (Figure 6). The position of the ICAM-1 molecule relative to the icosahedral symmetry axes of the virus is unambiguous (Kolatkhar *et al.*, unpublished results) and shows the receptor binding into the canyon (Figure 7). Each D1D2 molecule has an approximate dumbbell

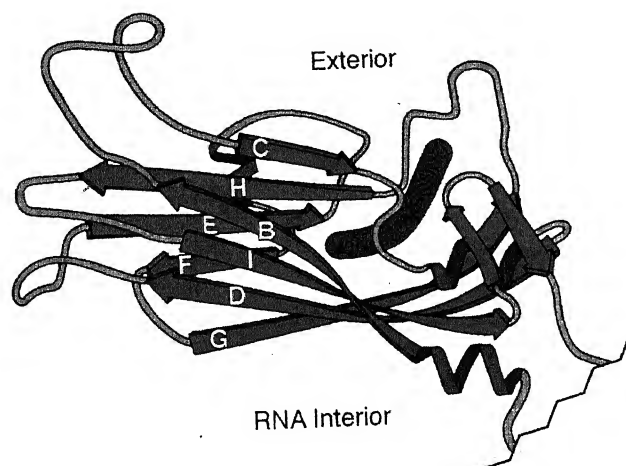


Figure 3. Schematic representation of the VP1 fold of HRV14. The folding topology of the two sheets 'BIDG' and 'CHEF' is the same in VP2 and VP3 as well as in most other viral capsid proteins. The binding site of antiviral WIN compounds within the hydrophobic interior of VP1 is also shown.

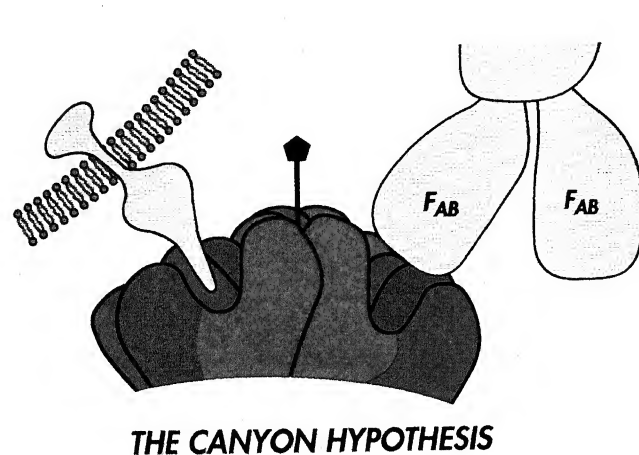


Figure 4. The presence of depressions on the picornavirus surface suggests a strategy for the evasion of immune surveillance. The dimensions of the putative receptor binding sites, the 'canyon', sterically hinder an antibody's (*top right*) recognition of residues at the base of the site, while still allowing recognition and binding by a smaller cellular receptor (*top left*). This would allow conservation of receptor specificity while at the same time permitting evolution of new serotypes by mutating residues on the viral surface, outside the canyon.

shape, consistent with the presence of two-domain structure. A difference map between the EM density and the 20 Å resolution HRV16 or HRV14 densities confirmed that the D1D2 molecule binds to the central portion of the canyon roughly as predicted by Giranda *et al.*⁴⁶. There are some small differences in orientation of D1D2 when complexed to HRV16 or HRV14 which may relate to the change in length of the VP1 BC loop forming the north rim of the canyon (Kolatkhar *et al.*, unpublished results). The D1D2 ICAM fragment is oriented roughly perpendicular to the viral surface and extends to a radius of about 205 Å. Its total length is about 75 Å.

Extensive structural similarity between D1D2 of ICAM-1 and CD4 was shown by means of a cross-rotation function between the known structure of D1D2 for CD4 (refs 40, 41), and the crystal diffraction data for ICAM-1 D1D2 (P. R. Kolatkhar and M. G. Rossmann, unpublished results). Thus, it seemed reasonable to use the known structures of CD4 for fitting the reconstructed density map (Figure 6), although there was slightly too little density for domain D1 and too much density for D2. A better assessment of the fit of domain D1 to the density was obtained by taking the predicted D1 structure of ICAM-1, including all side chains, and superimposing it onto the fitted C_α backbone of CD4. One major difference is that although domain D1 of CD4 resembles a variable, immunoglobulin-like domain with two extra β-strands, the ICAM-1 sequence is shorter and more like a constant C1 domain⁴⁶, although Berendt *et al.*⁴⁷ suggest that the topology might be like a constant C2 domain in which strand C is not part of either sheet

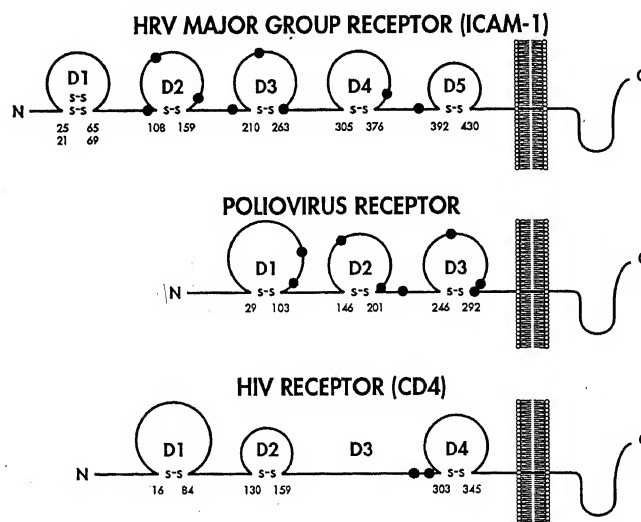


Figure 5. Schematic diagram of viral receptors. The relative size and distribution of immunoglobulin-like domains are shown. The black circles show the position of potential glycosylation sites. Numbers indicate the amino acid positions of Cys residues involved in predicted disulfide (S-S) bridges. [Reprinted with permission from Colonna¹⁰⁴. Copyright by Academic Press.]

region. This gives domain D1 of ICAM-1 a sleeker appearance, consistent with the observed difference density. The extra density in D2 (in the region farthest away from the virus) compared with domain D2 of CD4 is probably due to the four associated carbohydrate groups located in this region.

The footprint of ICAM-1 onto the HRV14 structure (Figure 8) correlates very well with Colonno's mutational studies of residues in the canyon which alter affinity of the virus to HeLa cell membranes⁴⁸. All the residues are part of the canyon floor and lie centrally within the footprint of the D1D2 molecule binding site. Similarly, there is excellent agreement between the ICAM-1 footprint and residues on the virus surface whose conformation is changed by antiviral agents⁴⁹⁻⁵¹.

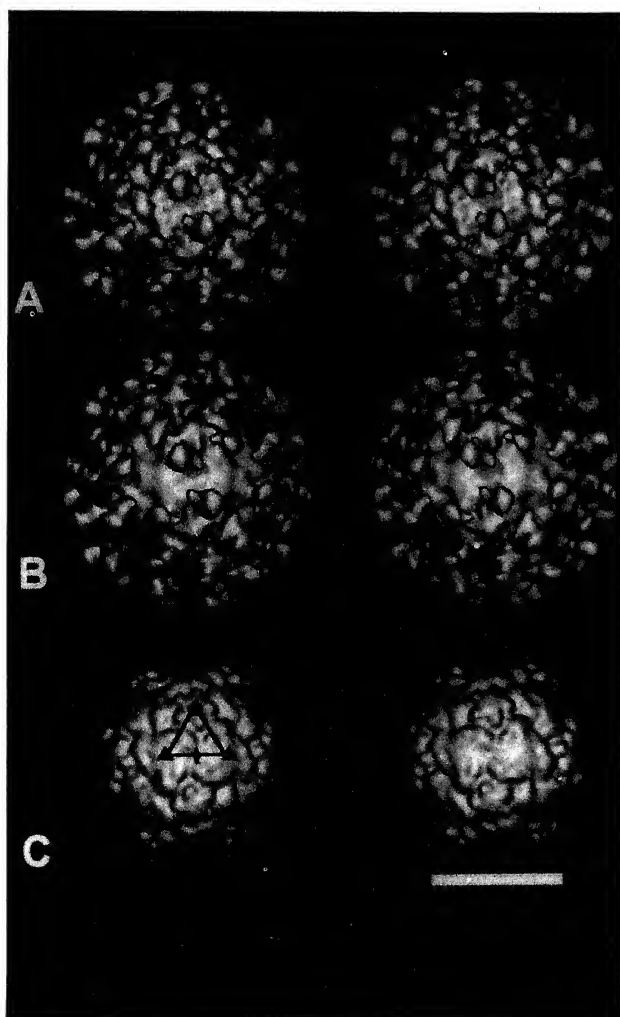


Figure 6. Stereo views of cryo EM image reconstructions of (A) HRV16 (green)–D1D2 (orange) and (B) HRV14 (blue)–D1D2 (orange) complex, viewed along an icosahedral two-fold axis in approximately the same orientation as in Figure 2. Both (A) and (B) show sixty D1D2 molecules bound to symmetry-equivalent positions in the canyons on the virion surface. (C) Shaded-surface view of HRV14 (blue), computed from the known atomic structure¹, truncated to 20 Å resolution.

Virus entry and uncoating

Productive viral uncoating requires that the RNA moves from inside the viral protein shell, through a cellular membrane, into the cytosol. Such displacement probably requires large conformational changes in the rhinovirus coat. For poliovirus or rhinovirus, acidification of endosomes, may be required for an infection to proceed normally as measured by either progeny virus production or cytopathic effects⁵²⁻⁵⁶.

Rhinovirus and poliovirus 149S infectious virions undergo several progressive transformations^{57,58} when bound to cells, which can be followed by sedimentation through sucrose gradients. The 149S virions are initially converted to 135 to 125S particles which have lost VP4 but retain RNA ('A-particles'). Subsequently, the RNA is released with the formation of 80S empty capsids as well as small capsid fragments.

The A-particles have a number of properties which suggest a role in virus entry. They have been shown to be hydrophobic and able to bind to liposomes^{59,60}. It has also been shown that the formation of poliovirus A-particles is associated with externalization of the N-terminus of VP1 and that removal of approximately 30 residues from the N-terminus of VP1 by proteolysis abolishes the ability of poliovirus to bind to liposomes⁶¹.

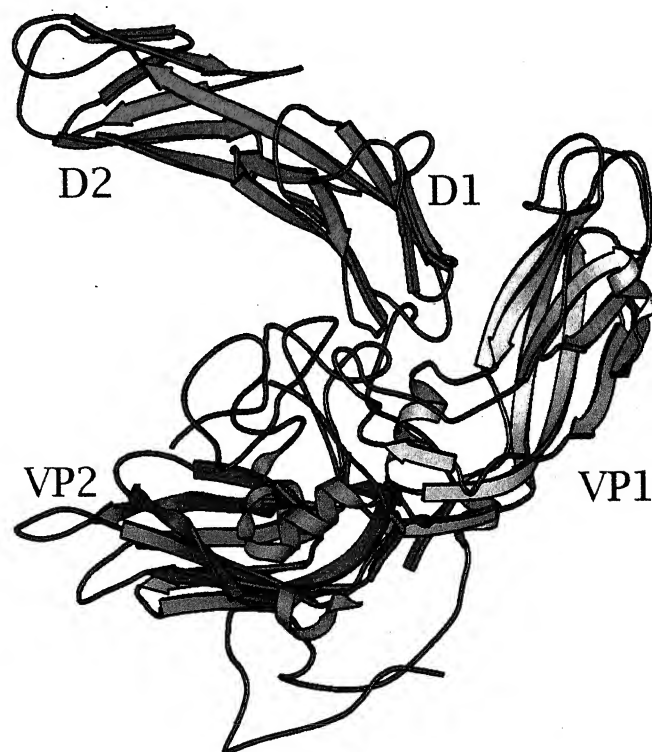


Figure 7. Structure of HRV16 VP1 (blue), VP2 (green) and part of VP3 (red) complexed with D1D2 of ICAM-1 (orange) modeled from the known structure of CD4.

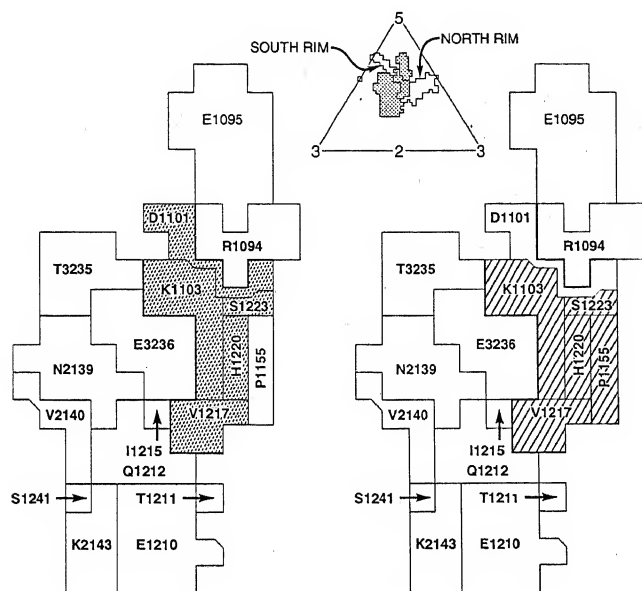


Figure 8. (Top) View of the icosahedral asymmetric unit bounded by adjacent five- and three-fold axes, outlining residues on the HRV14 surface. The limits of the canyon are shown, arbitrarily demarcated by a 138 Å radial distance from the viral center¹⁰⁵. Residues under the ICAM-1 footprint are stippled. Improved resolution of the electron density could only marginally alter the HRV residues at the virus-receptor interface. (Left and right) Enlarged view of the residues in the ICAM-1 footprint showing the residues (hatched areas) that, when mutated, affect viral attachment (right)⁴⁸, and the residues (stippled areas) altered in structure by the binding of antiviral compounds that inhibit attachment and uncoating (left)⁴⁹. [Reprinted with permission from Olson *et al.*⁴⁵. Copyright by the National Academy of Sciences.]

The sequence of the amino-terminal 23 residues of VP1 suggests that it could form an amphipathic α -helix and, thus, could promote interactions with lipid bilayers.

A-like particles can be generated under certain conditions *in vitro*^{60,62,63}. HRV14 incubated at pH 5–6, the pH likely to be found in endosomes, is converted to 135S A-particles. HRV14 incubated with soluble ICAM-1 is converted, through a virus-receptor complex intermediate, to 80S empty capsids, suggesting that receptor binding can destabilize the virion⁶⁰.

Since the conformational changes required for uncoating which occur on acidification are probably similar to those that occur on viral interaction with receptor, a structural determination of these changes could be useful. It has been possible to study the initial changes that occur in wild-type HRV14 crystals upon lowering the pH by using a very high intensity synchrotron X-ray source⁶⁴. This permitted the rapid recording of the diffraction pattern before the crystals completely disintegrated. It was found that an ion-binding site (Figure 9) on the icosahedral five-fold axes, the interior of the virus shell near the five-fold axes including the amino end of VP3, much of the ordered part of VP4 and the GH loop of VP1 all became disordered. Furthermore, the magnitude of the disorder increased as the time of acid exposure increased. The expansion of the β -cylinder and cation release, therefore, may be among the first events permitting eventual escape of VP4s, possibly along the five-fold axial channels. There are parallels to this process in the externalization of VP1 through

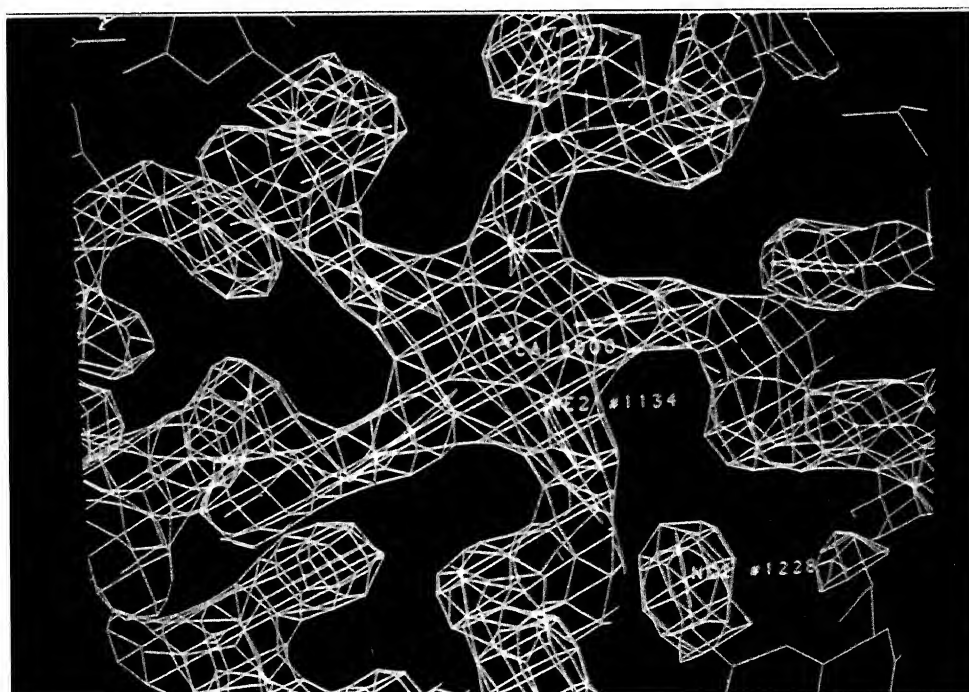


Figure 9. A putative Ca site in HRV16 with five His 1134 ligands (Hadfield *et al.*, unpublished results). Similar cation sites are found in CVB3 and HRV14. The ion comes off on acidification in HRV14.

the five-fold axial channels of canine parvovirus⁶⁵, and the ejection of single-stranded DNA through the five-fold ion channel of ϕ X 174 (refs 66, 67). An alternative proposal made by Fricks and Hogle⁶¹ based on mutational analyses and a comparison with properties of tomato bushy stunt virus⁶⁸ suggests that the first step in uncoating and the externalization of VP1 is a weakening of the contacts between protomeric units (Figure 2).

Inhibition of uncoating and the pocket factor

Capsid-binding, antiviral agents such as the 'WIN' compounds bind into a hydrophobic pocket in VP1 below the canyon floor. Not only do they inhibit attachment in HRV14 and other major group rhinoviruses, but they

also stabilize major and minor group rhinoviruses *in vitro* to acidification⁶⁹ and heat⁷⁰. HRV14 differs from other picornaviruses in that its pocket is empty in the native structure. For example, there is electron density in the homologous pockets of poliovirus Mahoney 1, poliovirus Sabin 3 and in a chimera of poliovirus 2 (refs 9, 12, 71). This density has been interpreted as a sphingosine or palmitate-like molecule because of the hydrophobic nature of the pocket and the polar environment at one end of the pocket. Similarly, the somewhat smaller electron density in the pocket of HRV1A (refs 13, 72) and HRV16 (ref. 18) has been tentatively interpreted as a fatty acid, eight or more carbon atoms long. A rather longer 'pocket factor' is found in this pocket for coxsackievirus B3 (CVB3) (J. K. Muckelbauer,

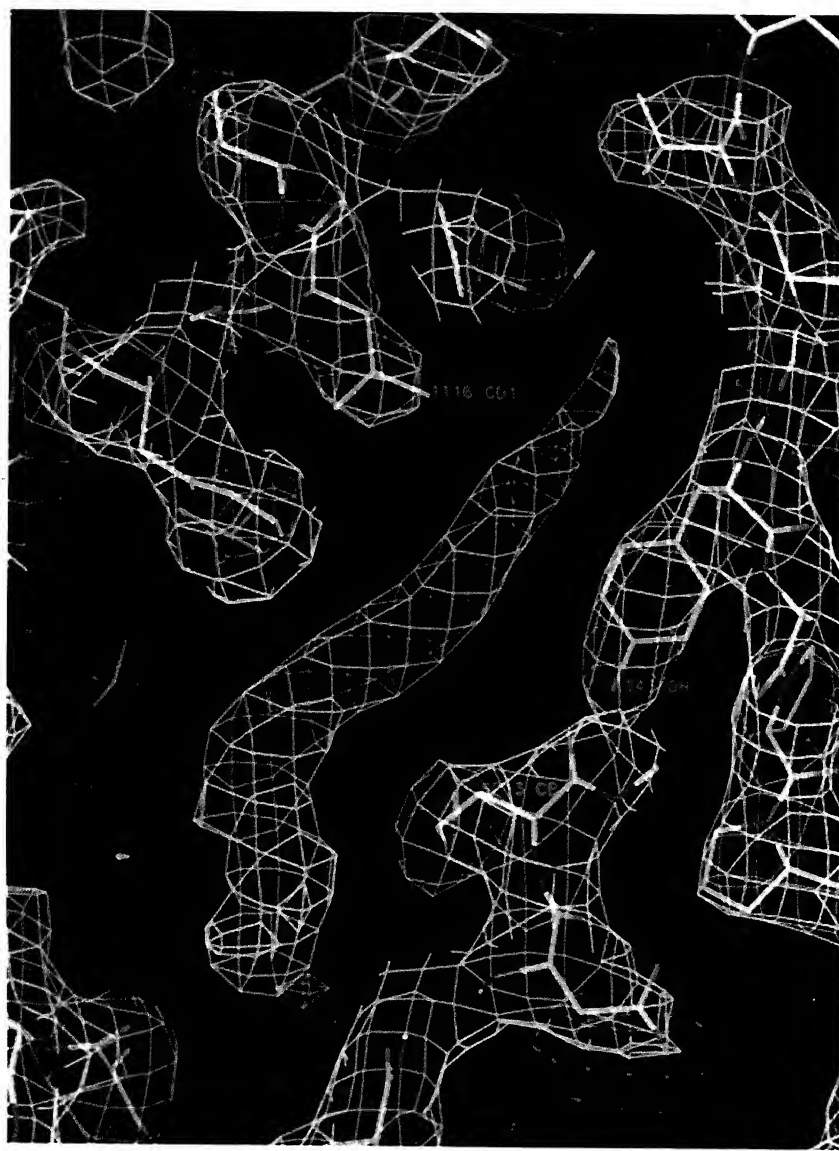


Figure 10. Electron density in the hydrophobic interior of VP1 corresponding to the site of binding of certain antiviral compounds (Figure 2) of coxsackievirus B3 (Muckelbauer *et al.*, unpublished results).

L. Tong, M. G. Rossmann and M. J. Kremer, unpublished results) (Figure 10). While it is possible that the pocket factor might be a small impurity picked up in the extraction procedure, with detergent or during crystallization with polyethylene glycol, these conditions differ greatly among the known structures. Smith *et al.*⁴⁹ imply, while Filman *et al.*¹² explicitly propose that the pocket factor could be cellular in origin and might regulate viral assembly and uncoating.

Binding of WIN compounds to HRV14 causes major conformational changes in the pocket and, hence, also in the canyon floor (the receptor attachment site). These changes were correlated to inhibition of attachment in the presence of the antiviral compounds^{50,51}. In contrast, in HRV1A (a minor receptor group virus) and polioviruses, where the WIN compounds merely displace the pocket factor without a correspondingly large conformational change, there is inhibition of uncoating but not of attachment. Preliminary results suggested that rhinoviruses of the minor receptor group exhibited no inhibition of attachment, whereas those of the major receptor group behaved like HRV14 for which attachment is inhibited.

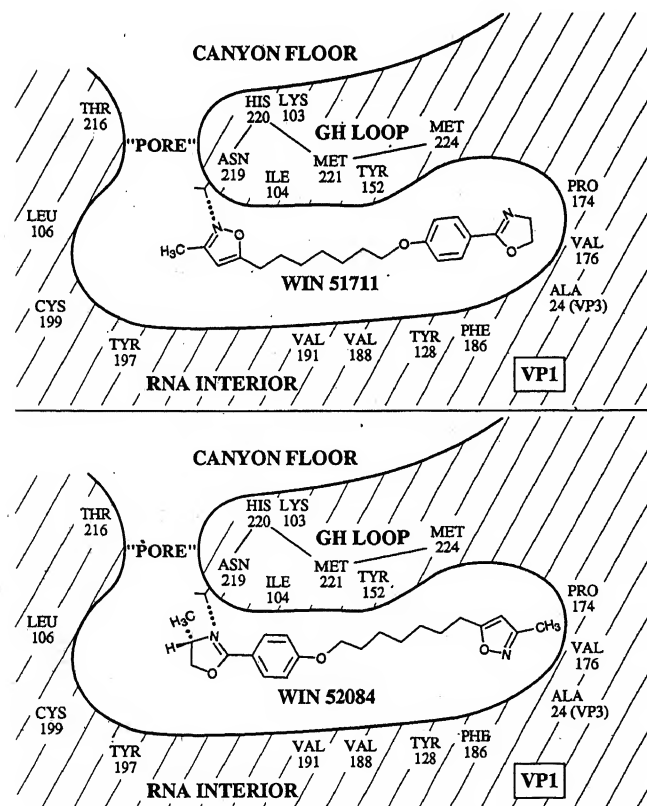


Figure 11. Schematic representation of the binding of the antiviral agents WIN 51711 and 52084 into a pocket underneath the canyon in HRV14. This causes enlargement of the pocket and conformational changes in the floor of the canyon, inhibiting attachment of the virus to HeLa cells in some cases, and also increasing the stability of the virus in all cases. [Reprinted with permission from Dutko *et al.*¹⁰⁶. Copyright by Springer-Verlag, New York.]

Thus, it was a surprise to find 'pocket factor' electron density in HRV16, causing the shape of the pocket to resemble that of the 'WIN filled' form of HRV14 (refs 13, 72).

In HRV1A and HRV16, the more active antiviral compounds tend to have an aliphatic chain less than or equal to five carbon atoms long⁷³, correlating with the available space within the binding pocket^{72,74,75}. In HRV14, the most active antiviral agents tend to be longer with seven-carbon aliphatic chains. For example, WIN 56291 has an aliphatic chain of only three carbons (compare Figure 11) and is equally active against HRV16 and HRV1A, but less active against HRV14. Thus, for each serotype there is an optimal drug size which displays the greatest activity and binding affinity^{74,75} and

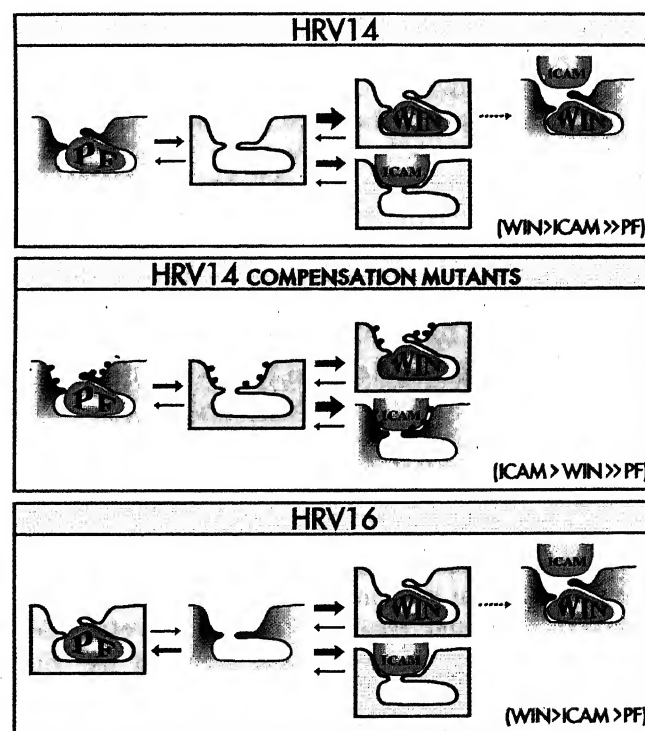


Figure 12. Conditions for inhibition of viral attachment by WIN compounds. Crystallographically and electron microscopically determined structures are in yellow and pink, respectively, while hypothetical structures are in gray. (Top) In wild-type HRV14, the pocket factor binds weakly and is not observed in crystallographic studies. When WIN compounds bind into the pocket, they deform the roof of the pocket which is also the floor of the canyon. This inhibits the attachment of the virus to the ICAM-1 receptor and, hence, presumably the binding affinity of WIN is greater than that of ICAM-1. When ICAM-1 recognizes the canyon floor, the putative pocket factor must be displayed by ICAM-1 and, hence, the binding affinity of ICAM-1 is greater than that of pocket factor. (Center) Drug-resistant compensation mutants of HRV14 cluster around the canyon walls and floor (•) and increase the affinity of ICAM-1 for the virus. Although WIN compounds can bind to the virus, they do not inhibit infectivity. Thus, the binding affinity of the mutant virus to ICAM-1 is greater than that of WIN. (Bottom) Wildtype HRV16 contains a pocket factor. This can be replaced by WIN compounds which inhibit attachment. Hence, in this case the affinity of HRV16 for WIN is greater than that of ICAM-1 which is greater than that of pocket factor.

best fills the volume of the pocket. It follows that the smaller pocket factors, which can be easily displaced by WIN compounds in HRV16 and HRV1A (refs 18, 72), bind with less affinity than the antiviral compounds. Nevertheless, the pocket factors seen in the electron densities remain in the pocket even after extensive dialysis of the virus sample. The WIN compounds have a binding constant comparable to their minimal inhibitory concentrations of $\sim 10^{-8}$ M (refs 70, 76).

The role of the pocket factor

When the antiviral binding pocket in HRV14 is filled with WIN compounds or fragments of WIN compounds that do not inhibit infectivity, there is an increase in the thermal stability of the virus^{77,78}, presumably as a consequence of placing a hydrophobic molecule into an internal hydrophobic cavity^{79,80}. Similarly, drug-dependent mutants of poliovirus require WIN compounds to maintain their stability⁸¹. The pocket factor may, therefore, be required to stabilize the virus in transit from one cell to another. However, the delivery of the infectious RNA into the cytoplasm must require a destabilizing step which might be effected by expulsion of the pocket factor during the receptor-mediated uncoating.

Since ICAM-1 binds to HRV14 and to HRV16 (Figure 12), the shape of the canyon for HRV16 should be similar to that in HRV14 when ICAM-1 binding occurs. As soluble ICAM-1 binds to purified HRV14, which does not contain any pocket factor, presumably the pocket is empty when ICAM-1 binds to HRV16. However, the structure of HRV16 shows the presence of a pocket factor in the purified virus¹⁸. Hence, it must be assumed that the pocket factor is displaced before the receptor can seat itself into the canyon. In essence, there are two competing equilibria: the binding of ICAM-1 and the binding of the pocket factor to the virus. Although the sites of binding of ICAM-1 and of the pocket factor are not the same, they are in close proximity and interfere with each other. The floor of the canyon is also the roof of the pocket for the pocket factor or WIN compounds. When ICAM-1 binds, the floor is depressed downwards, which is possible only when there is nothing in the pocket. Conversely, when there is a compound in the pocket, its roof is raised upwards. The displacement of the pocket factor *per se* does not cause the virus to fall apart. For instance, when HRV14 is crystallized it does not contain a pocket factor, and the complex of HRV16 with ICAM-1 is reasonably stable. Nevertheless, the absence of pocket factor increases the potential for disruption by lowered pH or by formation of the receptor-virus complex.

Presumably, the destabilization of the virus on cell attachment is made possible by the displacement of a sufficient number of pocket factors when the receptor

competes for the overlapping binding site. Progressive recruitment of receptors is then sufficient to trigger release of the VP4s. The terminal myristate moieties of VP4 and the exposure of the amino terminus of VP1 will permit entry through the cell membrane, possibly by creating a channel along the five-fold axes of the virus⁶⁴.

A class of HRV14 drug-resistant (compensation) mutants can be selected by growing the virus in the presence of antiviral WIN compounds. Such mutants occur at a frequency of about one per 10^4 virions. They have been shown to be mostly single mutations^{77,82} and six of the seven characterized to date are situated near the walls and floor of the canyon. WIN compounds bind into the pocket of these mutant viruses and deform the canyon floor in a similar manner to their effect on wild-type viruses (M. A. Oliveira, I. Minor, R. R. Rueckert and M. G. Rossmann, unpublished data). In some of these mutants, the affinity of ICAM-1 for the virus is enhanced (R. R. Rueckert, private communication; M. P. Fox, D. C. Pevear and F. J. Dutko, unpublished data). Thus, it is reasonable to conclude that ICAM-1 binds better to these mutant viruses than the WIN compounds (Figure 12, center).

Conclusions

The canyon hypothesis, which suggested that the receptor binding site can be hidden from immune surveillance in a 'canyon' on the surface of the capsid, has been verified for the major group of rhinoviruses. Mutational analyses have indicated that the canyon is also the receptor attachment site for poliovirus⁸³.

A virus must be stable in the extracellular environment during transit between hosts, but also must be destabilized once it has bound to or entered the host cell, shedding its protein coat to allow infection to proceed. In rhinoviruses and polioviruses, the need for reversible stabilization appears to be fulfilled by the binding of a small cellular aliphatic molecule, the 'pocket factor' into a hydrophobic pocket in VP1. In major group of rhinovirus serotypes, the binding site for ICAM-1, the virus receptor, overlaps with the binding site of the stabilizing pocket factor. Virus attachment is, therefore, a competition between two equilibria – (i) binding of the pocket factor into the pocket and (ii) binding of the receptor into the canyon. Provided that receptor competes successfully with the pocket factor, many pocket factors will be lost as receptor molecules are recruited, destabilizing the virus as a prelude for uncoating. Certain antiviral compounds also bind in the hydrophobic pocket, displacing the pocket factor. If the affinity of an antiviral compound for the pocket is higher than that of ICAM-1, the antiviral compound will prevent receptor attachment and uncoating. Drug escape mutations in VP1 that improve

binding affinity for ICAM-1 can shift this balance, overcoming the antiviral effect (Figure 12).

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Multidrug resistance: An emerging threat

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Multidrug resistance (MDR) has been the main cause of failure of cancer chemotherapy where it is defined as the tendency of tumour cells to exhibit simultaneous resistance to unrelated chemotherapeutic agents. MDR has been mainly associated with the overexpression of an ATP binding cassette (ABC) protein, P-glycoprotein. Research in the past decade has revealed that the MDR phenomenon is not restricted to mammalian cells but rather occurs throughout the evolutionary scale. Thus over hundred ABC proteins have been characterized in mammals, bacteria and yeast. This review briefly describes the advancement in this field and identifies the problems which have emerged due to MDR.

MULTIDRUG resistance, which is a major problem in medical and agricultural developments, is an emerging phenomenon observed in various organisms throughout the evolutionary scale. In agriculture, the control of resistance of plant pathogens towards natural plant defence toxins and other common fungicides, as well as the emergence of parasite-toxin resistant crops, are of major economic importance. In medicine, the problem of cancer is compounded by the acquisition of multidrug resistance (MDR) by human malignancies. MDR has been one of the principle causes of failure of cancer chemotherapy where it can be defined as the tendency of tumour cells in patients and cultured cells to exhibit simultaneous resistance to multiple chemically unrelated chemotherapeutic agents^{1,2}. The elucidation of the mechanism by which tumour cells develop resistance to toxic effects of potent chemotherapeutic agents has revealed a great deal about the process of drug uptake, metabolism and extrusion. This has also provided basic insights into cellular process such as regulation of gene expression and gene amplification¹⁻³. It has been shown that overexpression of certain ATP-binding cassette (ABC)-proteins in prokaryotes and eukaryotes is linked to drug resistance phenomenon⁴. The well characterized mammalian protein MDR1 (P-glycoprotein) is associated with the development of a drug-induced multidrug resistance phenotype in tumour cells¹⁻⁵. Further, overexpression of *Ldpgp A* from *Leishmania* is responsible for methotrexate and heavy metal resistance, and *Plasmodium Pfmdr* has been implicated in chloroquine resistance in the malarial parasite⁶⁻¹⁰. Likewise, bacterial erythromycin resistance in *Staphylococcus* is caused by *MsrA* overexpression,

and the ABC-protein *DrrAB* of *Streptomyces* appears to be daunomycin resistance determinant^{11,12}.

Drug resistance

In mammalian cells

Selective passage of specific molecules across membrane is the key to cell's survival which is achieved by specific membrane transporters. The importance of membrane transport is becoming even more apparent from genome sequencing projects where a majority (20-30%) of genes have been found to encode for membrane and particularly transport proteins¹³. It has been shown that there exists a limited number of transporter families where member proteins of a family are related to each other in sequence and in molecular mechanism and probably have a common evolutionary origin^{11,12,14}.

It is now clear that a major mechanism of MDR in mammalian cells involves the overproduction of a 170 kDa plasma membrane glycoprotein, P-glycoprotein^{1,5,15}. This protein appears to cause MDR via an ATP-dependent drug efflux mechanism, which prevents the intracellular accumulation of drugs to an effective cytotoxic concentration¹⁻³. P-glycoprotein is a member of super gene family of bacterial and eukaryotic transporter proteins (Table 1). The mammalian P-glycoproteins are encoded by small families of linked genes, two in humans, three in rodents. The human *MDR1* gene, the *mdr1* and *mdr3* genes of mice and the *pgp1* and *pgp2* of hamster encode related proteins which transport hydrophobic drugs^{1,3,16}.

Cloning and sequencing was a major step towards understanding the structure and function of P-glycoprotein. The sequence encoding P-glycoprotein revealed that it is a tandemly repeated molecule of about 1280 amino acids (~170 kD). Each half consisting of a large hydrophobic domain containing three pairs of putative membrane-spanning α -helices and a conserved hydrophilic cytoplasmic domain containing an ATP-binding site^{1,17-20}. It has been proposed that the 12 transmembrane domains associate to form a pore or channel through which P-glycoprotein actively effluxes drugs^{1,5}. *In vitro* mutagenesis of the putative ATP-binding sites suggests that both sites are required and these may functionally interact to affect drug efflux^{1,21}.

Although the mechanism of drug transport has not

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been defined, it is thought that direct binding of the drug to P-glycoprotein could be one of the essential steps¹. Extensive genetic manipulation involving deletion and insertion analyses of human MDRs has revealed that there are several coding regions which appear to have no effect on drug binding and specificity. However, there are several point mutations scattered throughout the gene which selectively alter drug specificity of the P-glycoprotein²¹. The drug specificity of MDR is a complex phenomenon which either requires a highly ordered structure or is affected by multiple independent parts of the protein molecule^{22,23}.

The ability of drugs and reversing agents to inhibit each other's binding to P-glycoprotein suggests that they

compete for common binding site(s)¹ (Table 2). Thus, one mechanism of MDR reversal by chemosensitizers and non-toxic drug analogues may be explained on the basis of competition for drug binding, which results in a decrease in efflux rate and a higher intracellular level of toxic drugs in MDR cells²¹. The P-glycoprotein recognizes a diverse group of substrates and shows different cross reactivity profiles¹⁻³. It is believed that a spontaneous mutation in P-glycoprotein gene, leading to altered drug specificity, may change the overall MDR profile^{1,21-25}.

That other mechanisms may also generate diversity in MDR phenotype has not been completely ruled out. In rodents, two different P-glycoproteins confer MDR

Table 1. Multidrug resistance pumps identified from microbes to man*

Organism	Proteins	Family	Function/substrate	Topology®
Prokaryotes				
<i>E. coli</i>	EmrE/MvrC	Major facilitator	Drug/H + transporters	12–14 TM helices
<i>Staphylococcus</i>	QacA	Major facilitator	Drug/H + transporters	
<i>Staphylococcus</i>	MsrA	Major facilitator	Drug/Antibiotics transporter	
<i>B. subtilis</i>	Bmr	Major facilitator	Tetra phenyl phosphonium	12–14 TM helices
Yeast (See Table 3)				
Protozoa				
<i>P. falciparum</i>	Pfmdr	ABC protein	Chloroquinine	12 TM helices
<i>L. donovani</i>	Ldmdr		Arsenite (?)	
Moulds				
<i>C. elegans</i>	Cepgp Ag	ABC protein	?	12 TM helices
Insects				
<i>Drosophila</i>	Mdr 49/50	ABC protein	?	12 TM helices
Plants				
<i>Arabidopsis</i>	Atpgp	ABC protein	?	12 TM helices
Mammals				
Hamster	Pgp1	ABC protein	Lipophilic drugs	12 TM helices
Mouse	Mdr1	ABC protein	Lipophilic drugs	12 TM helices
Man	Mdr1	ABC protein	Anticancer/lipophilic drugs	12 TM helices
Man	CFTR	ABC protein	Chloride channel	12 TM helices

*The table is compiled from refs 3, 11, 12, 53.

®Deduced from hydropathy analyses.

Table 2. Compounds which can interact with the multidrug resistance pump

Anticancer drugs	Other cytotoxic drugs	MDR-reversing agents	Cyclic and linear peptides
Daunorubicin	Colchicine	Verapamil	Gramicidin D
Doxorubicin	Emetine	Quinidine	Valinomycin
Mitoxanthrone	Ethidium bromide	Quinine	Yeast a-factor pheromone
Etoposide	Puromycin	Cyclosporin A	N-acetyl-leucyl-leucyl-norleucine
Teniposide	Podophyllotoxin	Forskolin	
Vinblastine		Azidopine	
Vincristine			
Actinomycin D			
Mitomycin C			
Taxol			
Topotecan			
Many others			

and differential expression of these genes probably could alter the stoichiometry of the individual isoform in the cell membrane, resulting in differences in profile of transported drugs²⁶. Furthermore MDR is a result of overexpression of P-glycoprotein gene which may be accompanied by the coexpression of very large stretches of flanking DNA. In Chinese hamster cell line, P-glycoprotein amplification has been shown to be over one mega base pair in size and at least six classes of genes have been found to be coamplified and overexpressed^{3,27,28}. It is, therefore, possible that overexpression of such linked gene may modify the drug resistance profile. Differences in drug resistance profile may also be the result of differences in post-translational modification of P-glycoprotein molecules itself^{3,26}. It has recently been found that P-glycoprotein is phosphorylated at both serine and threonine residues^{5,26}. It has been speculated that the extent of change in phosphorylation may modulate P-glycoprotein mediated drug transport mechanism. However, this remains to be confirmed. Study of P-glycoprotein glycosylation suggests that carbohydrate molecules do not affect drug resistance⁵. However, their role as modulators of P-glycoprotein function cannot be precluded.

In bacterial cells

When antibiotics like penicillin were discovered, some fifty years ago, they were treated as miracle drugs of the century. This scene has suddenly changed. We are now confronted with new resistant types of bacteria. Once bacteria have learnt a particular strategy to circumvent the toxic effect of an antibiotic, they exchange

the genetic information, without any species specificity, with other bacteria. As a result, now with every possible bacterial infection, resistance to antibiotic treatment is a common phenomenon. The cause of resistance is attributed to the amplification of bacterial MDR genes.

Most bacterial MDR come under major facilitators families (MFS) which include arabinose/H⁺ symporter of *Escherichia coli* and glucose facilitator of eukaryotes^{11,12}. The proteins of this family are similar to P-glycoprotein of eukaryotic cells but lack ATP binding domains and thus are not classified as ABC proteins. MFS have 12 transmembrane α -helical domains and use proton motive force as a source of energy. QacA is one of the first MDR proteins identified in bacteria. *Staphylococcus* acquires resistance to the quaternary ammonium compounds (QacA) used in antiseptics. QacA is a membrane pump which effluxes out several drugs in a proton motive force dependent manner^{11,29}. *emrA* and *emrB* are the two genes coded by *E. coli* which confer resistance to uncouplers (CCCP) and other antimicrobial agents^{11,30}. Interestingly, EmrB protein is homologous to QacA. EmrA, on the other hand, is homologous to HlyD (a component of *E. coli* hemolysin efflux pump) albeit to a lesser degree. The function of these proteins is to form a channel between the inner and outer membrane (Figure 1). In case of hemolysin pump, HlyB is the actual pump while HlyD and a porin (TolC) are needed to form a channel to allow the passage of the peptide outside the cell. Thus, the topological design of EmrA-EmrB could be the same as that of hemolysin pump¹² (Figure 1).

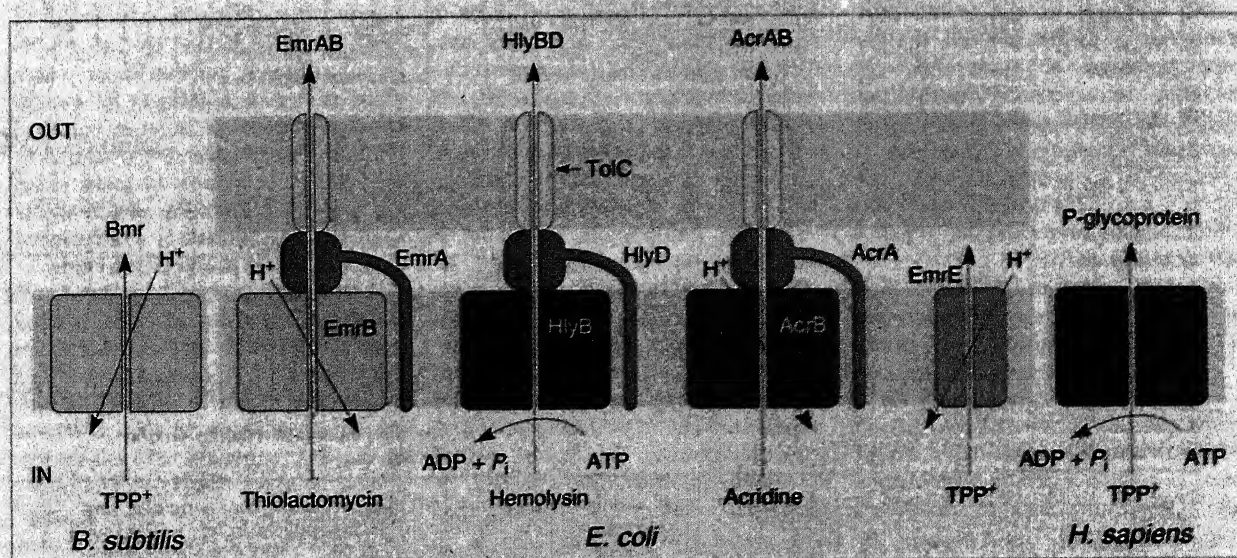


Figure 1. Topology of bacterial and human multidrug resistance. For comparison, the use of same colour indicates the homologous proteins in different MDR complexes. Reproduced from ref. 2 with permission.

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In yeast cells

Multidrug resistance phenomenon is not restricted to mammalian or microbial cells. Host of genes homologous to MDR have been identified in yeasts during the past three decades. Yeast shares similarity in structural and functional organization with higher eukaryotes and is amenable to genetic manipulations and thus, serves as an excellent model for unravelling eukaryotic pathways of MDR. The studies involving MDR in yeasts have got further impetus since some yeast species are also pathogenic to plants and humans. Already about 25 genetic determinants associated with multidrug resistance

(pleiotropic drug resistance, PDR in yeasts) have been characterized in *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and *Candida albicans*^{31,32}. The gene products encoded by these yeasts fall into three classes of proteins: ABC, MFS and transcription regulators (Table 3).

The *PDR5* gene was cloned as a multicopy plasmid borne DNA fragment capable of conferring pleiotropic drug resistance (PDR)^{33,34}. The gene codes for a polypeptide of 1511 amino acid residues with calculated mol. wt of 170.4 kD. PDR5 protein is predicted to contain twelve 'integral' transmembrane spans gathered in two groups of six contiguous membrane spans. Each hydro-

Table 3. Yeast proteins of multidrug resistance family

Yeast	Protein	Substrates	Membrane topology/function
<i>S. cerevisiae</i>	PDR5/STS1/YDR1	cyh, chl, ery, amy, sts, flu, smm.	ABC membrane protein. (NBD-TM)2
<i>S. cerevisiae</i>	SNQ2	4-NQO, MNNG, flu, sts, tri.	ABC membrane protein. (NBD-TM)2
<i>S. cerevisiae</i>	STE6	Val	ABC membrane protein. (TM-NBD)2
<i>S. cerevisiae</i>	YCF1	Cd	ABC membrane protein. (TM-NBD)2
<i>S. pombe</i>	pmd1	lep, cyh, val	ABC membrane protein. (TM-NBD)2
<i>C. albicans</i>	CDR1	cyh, chl, mic, amy	ABC membrane protein. (NBD-TM)2
<i>S. cerevisiae</i>	ADP1	—	ABC membrane protein. (NBD-TM)
<i>S. cerevisiae</i>	YKL741	—	ABC membrane protein. (TM-NBD)
<i>S. cerevisiae</i>	MDL1	—	ABC membrane protein. (TM-NBD)
<i>S. cerevisiae</i>	MDL2	—	ABC membrane protein. (TM-NBD)
<i>S. cerevisiae</i>	Ssh1	—	ABC membrane protein. (TM-NBD)
<i>S. cerevisiae</i>	Ssh2	—	ABC membrane protein. (TM-NBD)
<i>S. pombe</i>	HMT1	heavy metals (Cd)	Vacuolar. (TM-NBD)
<i>S. cerevisiae</i>	ATM1	—	ABC membrane protein. (TM-NBD)
<i>S. cerevisiae</i>	ATR1/SNQ1	atr, 4-NQO	Major facilitator
<i>S. cerevisiae</i>	YCL069w	—	Major facilitator
<i>S. cerevisiae</i>	YCL023c	—	Major facilitator
<i>S. cerevisiae</i>	YCL070c	—	Major facilitator
<i>S. cerevisiae</i>	YKR105c	—	Major facilitator
<i>S. cerevisiae</i>	YKR106w	—	Major facilitator
<i>C. albicans</i>	Ben ^r	ben, met	Major facilitator
<i>C. maltosa</i>	Cyh ^r	cyh	Major facilitator
<i>S. pombe</i>	car1	aml	Major facilitator
<i>S. cerevisiae</i>	PDR1	cyh, chl, oli, nys, ner, muc etc.	Transcription regulator
<i>S. cerevisiae</i>	PDR3	muc, chl, cyh, oli, tet, ner.	Transcription regulator
<i>S. cerevisiae</i>	yAPI/PDR4	Cd, Zn, cyh, tre, smm, 4-NQO,	Transcription regulator
	SNQ3/PAR1	phe, MNNG, nin	
<i>S. cerevisiae</i>	CAD1/YAP2	Cd, Zn, phe	Transcription regulator
<i>S. pombe</i>	pap1	sts	Transcription regulator
<i>S. cerevisiae</i>	PDR7	cyh, smm	—
<i>S. cerevisiae</i>	PDR9	cyh, smm	Transcription regulator
<i>S. cerevisiae</i>	RPD1	cyh	Transcription regulator
<i>S. cerevisiae</i>	RPD3	cyh	Transcription regulator
<i>S. cerevisiae</i>	YGL022	cyh, smm	—
<i>S. cerevisiae</i>	PDR6	cyh, bor, hygB	—
<i>S. cerevisiae</i>	PDR8	oli, smm	—
<i>S. pombe</i>	sts1	cyh, sts, caf, chl, divalent cation	—
<i>S. cerevisiae</i>	cpr	van	Soluble
<i>S. cerevisiae</i>	HOM3	bor	Soluble
<i>S. cerevisiae</i>	AMY1	amy	—
<i>S. pombe</i>	RIM-C	cyh	Soluble, ribosomal binding protein
<i>S. cerevisiae</i>	ZRC1	Zn, Cd	Transporter

Drugs are abbreviated as follows: atr, aminotriazole; amy, antimycin; aml, amiloride; ben, benomyl; bor, borrelidin; caf, caffeine; chl, chloramphenicol; cyh, cycloheximide; ery, erythromycin; flu, fluphenazine; hygB, hygromycin B; lep, leptomycin; mic, miconazole; muc, mucidin; nin, 1-nitroso-2-naphthol; MNNG, *N*-methyl-*N'*-nitrosoguanidine; 4-NQO, 4-nitroquinoline *N*-oxide; ner, neutral red; met, methotrexate; oli, oligomycin; phe, 1-10-phenanthroline; smm, sulfomethuron methyl; sts, staurosporine; tet, tetracycline; val, valinomycin; van, vanadate; tri, triaziquone; tre, trenimon. Other abbreviations are: NBD, nucleotide binding domain; TM, transmembrane region; ABC, ATP-binding cassette; (NBD-TM)2, NBD precedes TM and vice versa and has 2 halves. The table is compiled from refs 31, 41, 42.

phobic domain follows a hydrophilic region including a predicted ATP-binding cassette (ABC). Thus, PDR5 seems to have duplicated structure, consisting of two halves each composed of one hydrophilic and a hydro-

phobic domain^{34,35} (Figure 2). The two similar ABC domains of PDR5 are conserved within a large super family of transport proteins³⁵.

A sequence alignment of entire protein in databanks revealed homology between PDR5 and other members of the ABC-transporters superfamily. The best comparison was obtained with yeast ADP1, pheromone transporter STE6, *Drosophila* white and brown eye pigment transporter, bacteria hemolysin secretion protein B, mouse MDR1, rat major histocompatibility complex Mtp1 and most importantly with cystic fibrosis protein CFTR in humans. The region of homology is mainly localized on ABC cassette³⁴ (Figure 2). Recent evidences for the modular structure of a four-domain ABC transporter have been provided by domain dissection analysis of the yeast STE6 transporter^{36,37}. The two halves of the molecule were shown to be able to support, jointly, 'a' factor transport activity³⁸.

A *PDR5* homologue *CDR1* has recently been cloned and characterized in a pathogenic yeast, *Candida albicans*, by functional complementation of a *PDR5* null mutant of *S. cerevisiae*. The nucleotide sequence of *CDR1* revealed that, like *PDR5*, it encodes a putative membrane pump belonging to ABC superfamily³⁹ (Figure 2). Fresh evidences from our group suggest that there are several homologues other than *CDR1* in *C. albicans* which display cross resistance pattern different from *CDR1* and *PDR5*^{39,40}. Benomyl resistant (*Ben^r*) gene of *C. albicans* has also been shown to encode a putative membrane pump which belongs to MFS family⁴¹. The characterization of *CDR1*, *Ben^r* and identification of several other multidrug resistance genes from a pathogenic yeast could pave the way for tackling drug resistance in *Candida* and for the development of effective anti-*Candida* drugs. Recently, the field of drug resistance in pathogenic fungi has generated considerable interest because of spread of AIDS where *Candida* infections are most predominant.

A few ABC proteins have also been characterized in a fission yeast, *S. pombe*. HMT1 is a duplicated ABC protein associated with the vacuolar membrane and most similar to mammalian glycoprotein. Overexpression of the *HMT1* was correlated to enhanced heavy metal tolerance⁴². The *PMD1* encodes a half ABC protein (comprising of six transmembrane segments) homologous to *MDR1* and *STE6*. Overexpression of *PMD1* confers resistance to leptomycin B, cycloheximide and valinomycin³¹. HBA2, another ABC protein that confers resistance to brefeldin A and other drugs, has recently been identified in *S. pombe*⁴³.

The two pleiotropic drug resistance loci, *PDR1* and *PDR3*, were found to encode homologous transcription factors belonging to the family containing a 'Zinc 2 Cysteine 6' co-ordination complex in the DNA binding domain³². The *PDR1* gene product was shown to modulate

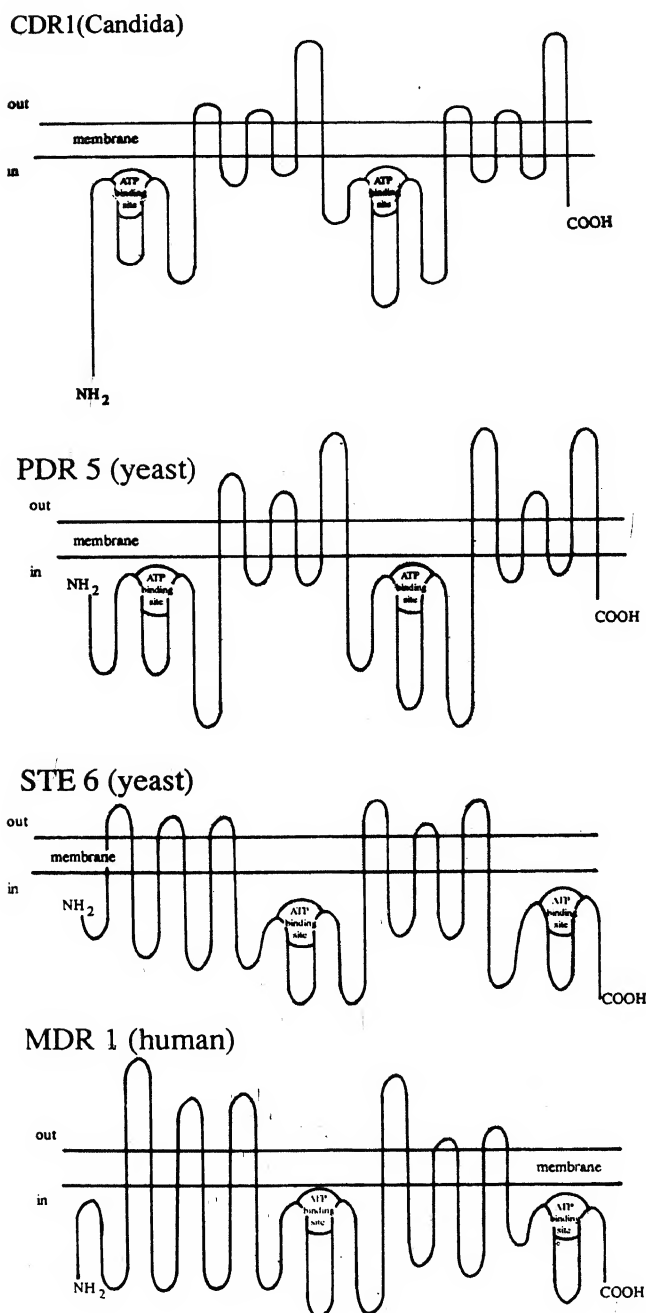


Figure 2. Predicted structure of the CDR1, PDR5, STE6 and MDR1 proteins. The CDR1 and PDR5 proteins are predicted to be composed of two repeated halves, each comprising of one hydrophilic domain followed by a hydrophobic domain. Two hydrophilic domains are cytoplasmic (IN) and each contains one ATP-binding site. The two hydrophobic domains are considered to be spanning the membrane. The sequence of domain inversion between CDR1 and STE6 can be seen.

the expression of multidrug resistance genes, such as *PDR5* and *STE6*, and also affect the estradiol levels^{32,44}. The fact that estrogen molecules are also substrates in the yeast *PDR* pathway, may provide a link between drug resistance and hormone tolerance³². The uncovering of regulatory elements, like *PDR1*, *PDR3*, etc. in yeast^{45,46}, might provide the basis for unravelling related circuits of control in human multidrug resistance.

Physiological role of P-glycoprotein

The availability of various sequences of P-glycoprotein genes of different species has allowed a comparison between different genes, both within a species and among different species. The comparison has shed some light on the evolution of P-glycoprotein and the organization of its gene. The similar organization of coding sequences and intervening sequences in different genes from the same species indicate that the internal duplication of the ancestral gene occurred prior to the formation of multigene family. The organization of homologous members of the multigene family in different mammalian species suggests that the formation of a multigene family preceded the divergence of species³. The evolutionary relation and conserved structure of P-glycoprotein leads to questions like: what is the physiological function of such proteins?

Expression of P-glycoprotein is cell and tissue-specific^{2,47,48}. Therefore, the tissue distribution may help to identify the physiological role of P-glycoprotein as a transporter. But given the specific and complex pattern of expression, it has been difficult to imagine a single class of physiological substrate². Therefore, it has been suggested that P-glycoprotein plays a diverse role in transport²³. However, as of now there are only a few examples from higher eukaryotes where the physiological role of MDR proteins has been identified (Table 4).

Mechanisms of MDR

Multidrug transporter (ABC type) does not function as a simple transmembrane transport system which effluxes out drugs from cytoplasm to extracellular space. Most of the substrates of pump are hydrophobic and thus tend to partition in nonpolar environment in preference to aqueous phase. Indeed, the data also suggest that anticancer anthracyclines, rhodamine-123 are predominantly localized in the plasma membrane and intracellular membranous structures in addition to their targets. The spectrum of drugs handled by the transporter suggests that a simple model of substrate recognition may not be correct. At least one transporter of ABC family CFTR (cystic fibrosis transmembrane regulator) is also a Cl⁻ channel⁴⁹⁻⁵¹. Therefore, a need to have a model

of P-glycoprotein transporter which encompasses all the conflicting observations has been realized⁵².

According to the most accepted model, drugs are removed by the transporter directly from the plasma membrane (lipid bilayer), thus, drugs are thrown out and are unable to reach the cytoplasm². Conceptually, the multidrug transporter works as a 'hydrophobic vacuum cleaner' which removes drugs from the membrane. The mechanism of energy transduction during drug transport is, of course, not clear. The nucleotide-binding domains of P-glycoprotein and constitutive ATPase activity therein do suggest a role of nucleotide hydrolysis. Some evidences also suggest that the drug transporter could be an enzyme 'flippase' which would bind the drug from the inner leaflet and flips it to the outer leaflet from where the drug diffuses out to extracellular space or the pump could behave like a moving 'water-wheel' or 'escalator', which expels all membrane constituents of approximately similar size (molecular weight) and shape, with little substrate specificity. There is still no unifying model which could include all conflicting observations of drug transport^{1,2,26} (Figure 3). But the recent suggestion of chloride channel activity associated with the multidrug transporter is consistent with the idea that the net positive charge (proton) accompanies drugs out of the cell and this may require an anion channel to maintain electric neutrality².

Future perspective

In the beginning a specific mechanism of antibiotic resistance was thought to be more important. Thus attempts were made to produce more effective antibiotics by modification of specific groups of antibiotic molecules in order to make them inert as potential substitute for commonly occurring antibiotic inactivating enzymes. However, the presence of more generalized mechanism of multidrug resistance has compelled the scientists to evaluate this strategy. As a result, several new drugs with new targets are in the pipeline and may hit the market in couple of years' time. Since these new drugs hit new targets it is hoped that bacteria will take still longer to learn to destroy them. There is also a need to obtain more knowledge about the substrate-binding process of these transporters. A possible approach would be to increase the spontaneous influx of drugs by making them sufficiently lipophilic so that efflux can be counter balanced by rapid influx. Indeed, it will be a major challenge for the pharmaceutical industry because some of the multidrug efflux systems seem to pump out almost any amphiphilic compound.

In plant pathogens, P-glycoprotein may be responsible for the secretion of fungal pathogenesis factors or toxic plant defence products playing a crucial role in plant-pathogen interaction. Understanding of the role of P-

glycoprotein in these processes would open new ways for indirect control of plant pathogens by interference with the plant-pathogen interaction. This particular area is still at its infancy. In this regard, recent cloning of a MDR homologue in *Arabidopsis thaliana* and identi-

fication of efflux pumps in pathogenic fungi of plants are interesting developments⁵³⁻⁵⁵.

In mammalian cells, where numerous approaches to reverse or modify MDR are currently being investigated, two important problems need to be re-emphasized:

Table 4. Some of the ABC-proteins with known substrates

Species	Protein	Substrate	Function
Bacteria			
<i>Salmonella typhimurium</i>	Opp ABCDF	Oligopeptides	Import
<i>Streptococcus pneumoniae</i>	Ami ABCDEF	Oligopeptides	Import
<i>Bacillus subtilis</i>	Opp (Spo K)	Oligopeptides	Import
<i>E. coli</i>	Dpp	Dipeptides	Import
<i>Bacillus subtilis</i>	Dci A	Dipeptides	Import
<i>S. typhimurium</i>	His JQMP	Histidine	Import
<i>E. coli</i>	His JQMP	Histidine	Import
<i>E. coli</i>	Mal EFGK	Maltose	Import
<i>S. typhimurium</i>	Mal EFGK	Maltose	Import
<i>Enterobacter aerogenes</i>	Mal EFGK	Maltose	Import
<i>E. coli</i>	Ugp ABCE	Gly-3-Phosphate	Import
<i>E. coli</i>	Ara FGH	Arabinose	Import
<i>E. coli</i>	Rbs ACD	Ribose	Import
<i>E. coli</i>	Gln HPQ	Glutamine	Import
<i>S. typhimurium</i>	Pro U (VWX)	Glycine-betaine	Import
<i>E. coli</i>	Pro U (VWX)	Glycine-betaine	Import
<i>E. coli</i>	Liv HMGF (JK)	Leu-Ile-Val	Import
<i>E. coli</i>	Pst ABC	Phosphate	Import
<i>Pseudomonas stutzeri</i>	Nos DYF	Copper	Import
<i>E. coli</i>	Chl JD	Molybdenum	Import
<i>E. coli</i>	Cys PTWAM	Sulphate-thiosulphate	Import
<i>E. coli</i>	Btu CDE	Vit. B ₁₂	Import
<i>E. coli</i>	Fhu BCD	Fe ³⁺ -ferrichrome	Import
<i>E. coli</i>	Fec BCDE	Fe ³⁺ -dicitrate	Import
<i>S. marcescens</i>	Sfu ABC	Fe ³⁺	Import
<i>Streptomyces fradiae</i>	Tlr C	Tylosin	Export
<i>Agrobacterium tumefaciens</i>	Occ JQMP	Octopine	Import
<i>E. coli</i>	Hly B	Hemolysin	Export
<i>Pasturella</i>	Ltk B	Leukotoxin	Export
<i>E. coli</i>	Cva B	Colicin V	Export
<i>Erwinia chrysanthemi</i>	Prt D	Proteases	Export
<i>Bordetella pertussis</i>	Cya B	Cyclolysin	Export
<i>Streptococcus</i>	Com A	Competence factor	Export?
<i>Haemophilus influenzae</i>	Bex AB	Capsule polysaccharide	Export
<i>E. coli</i>	Uvr A	-	DNA repair
<i>Rhizobium leguminosarum</i>	Nod I	-	Nodulation
Cyanobacterium			
<i>Anabaena</i>	Het A	-	Differentiation
<i>Synechococcus</i>	Cys A	Sulphate	Import
Yeast			
<i>S. cerevisiae</i>	STE 6	α -mating factor	Export
<i>S. cerevisiae</i>	EF-3	-	Translation
Protozoa			
<i>Leishmania</i>	Idpgp A	Heavy metals	Export
Insects			
<i>Drosophila</i>	white-brown	Eye pigments	Transport
Plants			
Liverwort chloroplasts	Mbp X	?	Transport?
Mammals			
Mouse	CFTR	Chloride	Channel
Man	CFTR	Chloride	Channel
Man	mdr 3	?	Flippase?

*Modified from ref. 11 with permission.

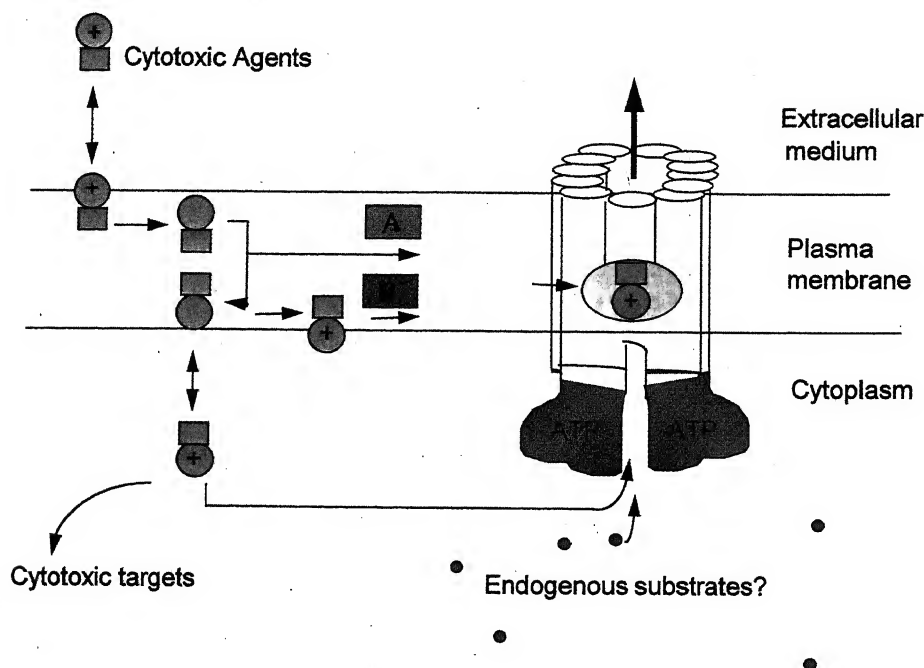


Figure 3. A model of MDR transporter. The drug is probably detected by the transporter within the lipid bilayer. Both uncharged (A) and charged (B) species of drugs could be the substrate for the transporter. The blue-coloured domains of protein indicate the ATP-binding sites. The red molecules are drugs and green molecules are putative physiological substrates for the transporter.

(i) MDR is unlikely, if ever, to be solely due to P-glycoprotein-mediated resistance, and (ii) P-glycoprotein is expressed by a very wide range of normal, noncancerous tissues as well. In the first case, therefore, prospective clinical protocol aimed at circumventing MDR may have to encompass more than just anti P-glycoprotein therapy. In the second case, successful anti-MDR therapy will probably have to be restricted to P-glycoprotein expressing tumour cells, to prevent unknown potentially deleterious consequences of inhibiting P-glycoprotein action in the normal healthy tissues.

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RESEARCH ARTICLE

Evidences of Late Quaternary neotectonic activity and sea-level changes along the western continental margin of India

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The offshore data on sea-level changes along the western margin of India have been reviewed and evidences of Late Quaternary neotectonic activity and subsidence are documented, based on the diagenetic textures of limestones from deeper submarine terraces and from the Fifty Fathom Flat off Saurashtra-Bombay, authigenic clays from the Kerala continental margin and onshore data. Offshore sea-level data relative to the eustatic sea-level show about 40 m subsidence sometime in the Holocene. Existing sea-level curves may not reflect the true sea-level changes. As there are several gaps in the data base, it is suggested that more systematically collected offshore data is an immediate requirement to chart the accurate sea-level changes and construct a regional sea-level curve for the Late Quaternary.

SEA-LEVEL change in a given area is governed by eustatic sea-level fluctuations and local factors such as land

movements by tectonic and isostatic adjustments and geoidal variations^{1,2}. The construction of regional sea-level curve is therefore essential and important in understanding the implications of sea-level changes. Kale and Rajaguru³ and Hashimi *et al.*⁴ constructed sea-level curves for the Late Quaternary for the western continental margin of India. These curves differ distinctly from one another and also with the eustatic sea-level curve of Fairbanks⁵ (Figure 1). The authors used some estimated³ and inferred ages⁴ for making sea-level curves. Incidentally, the actual radiocarbon dates of the samples from the western offshore (outer shelf and slope) (Figure 2) are younger than those at corresponding depths on the eustatic sea-level curve (Table 1) and thus plot away from all the above curves (see Figure 1). This may be due to neotectonism which was not considered in preparing the sea-level curves. In this article we provide evidences of Late Quaternary neotectonism along the western margin of India and reassessment of existing

Deeper terrace limestones

Rao and Veeraya⁶ reported 1.5 to 2.0 km wide submarine carbonate terraces at 130, 145 and 170 m water depths on the continental slope off Saurashtra–Bombay (Figure 3). These terraces lie below the eustatic sea-level low (–120 m)⁵ during the Last Glacial Maximum (LGM). The radiocarbon dates and diagenetic textures of the limestones from 130 m terrace indicate that these sediments were cemented into limestones at intertidal conditions at about 11,890 years BP. The eustatic sea-level was, however, at –90 m at about 12,000 years BP (ref. 5). Transportation of limestones from shallow shelf

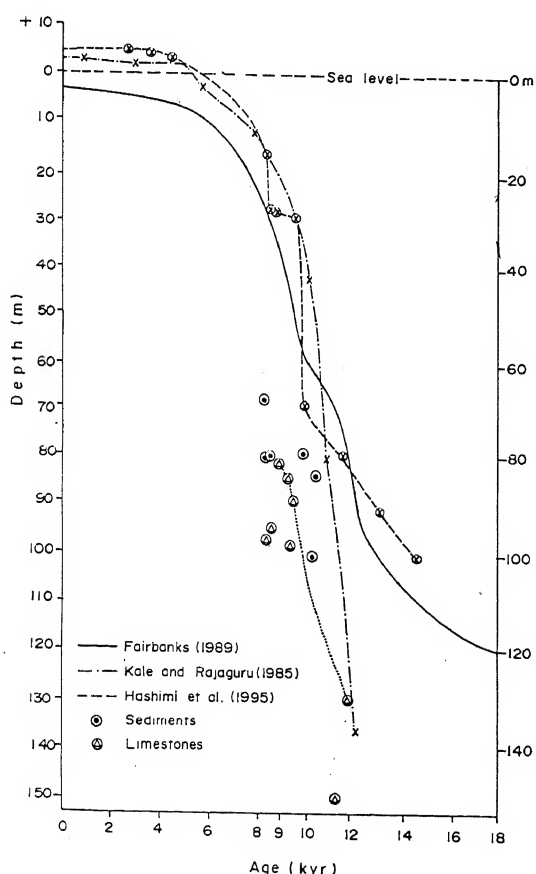


Figure 1. Sea-level curves and plot of radiocarbon dates of the samples from the western offshore. Note that the ages of the samples plot away from the curves. Fairbanks curve (ref. 5) is for the Barbados Islands; the ages of the coral samples used for the curve were corrected with the uplift data of the Barbados and for local sea water (Fairbanks sea-level curve is being used as a reference eustatic sea-level curve by several workers all over the world). The dotted line may represent sea-level change between 12,000 and 9,000 years BP.

depths can be precluded, because the terraces on landward are covered by 15–20 m thick sediments (Figure 3) and the ages of samples from the adjacent shelf are younger by about 2,000 years and nowhere else are these ages found (see Figure 2). It is therefore suggested that these deeper terraces on the Saurashtra–Bombay margin might have been positioned at water depths < 120 m during the LGM and subsided to the present position sometime after 12,000 years BP.

Radiocarbon dates and their comparison. The age of the aragonite sands and limestones on the carbonate platform ranges from 10,400 to 8,340 years BP and 9,200 to 8,465 years BP, respectively (see Table 1; Figure 2). There is no relationship between ages of the samples and their depth of occurrence and the sediments having younger ages occur even at greater depths. Further, the sediments and limestones with an age difference of about 2,000 years occur in the vicinity on the platform.

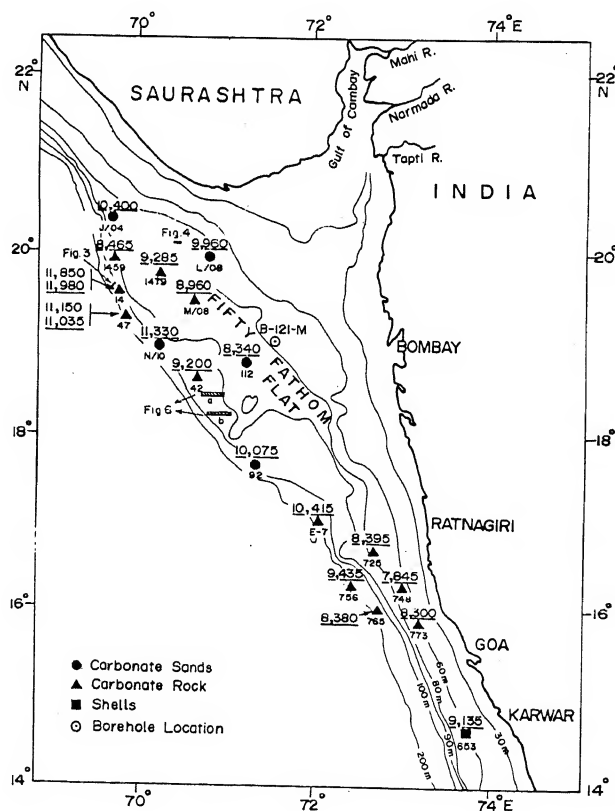


Figure 2. Sample location map of west coast of India, numbers underlined are radiocarbon ages. The location of deeper submarine terraces (Figure 3), *Halimeda* bioherms (Figure 4) and sand ridges (Figure 6) are also shown here.

Table 1. Available radiocarbon dates on surficial carbonate samples from the western continental margin of India (see Figure 2 for sample location)

Sample no.	Nature of sample	Depth (m)	Age (years BP) and reference	Age (approx.) on the eustatic sea-level curve ⁵ for the corresponding depth
47	Algal-pelletal l.st.	150	11,150 ± 130 (refs 8, 9)	—
E-7	Algal-bryozoan l.sts.	180*	10,415 ± 250 (refs 8, 9)	—
775	Algal nodules	70–150*	11,040 ± 135 (ref. 34)	—
			7,500 ± 200 (ref. 35)	
14	Beachrock	130	11,980 ± 185 (ref. 6)	—
			11,850 ± 210	—
42	Oolitic l.st.	98	9,200 ± 135 (refs 8–10)	12,500
M/08	Oolitic l.st.	82	8,960 ± 200 (refs 8–10)	11,850
L/08	Oolites	80	9,960 ± 160 (refs 8–10)	11,600
J/04	Oolites	80–90	10,400 ± 300 (ref. 9)	11,900
1459	Halimeda l.st.	95	8,465 ± 125 (ref. 15)	12,350
1479	Halimeda l.st.	85	9,285 ± 180 (ref. 15)	11,900
112	Pelletal sands	80	8,340 ± 185 (ref. 15)	11,600
92	Pelletal sands	100	10,075 ± 250 (ref. 15)	12,700
653	Shells	58	9,135 ± 130 (ref. 10)	9,850
725	Algal nodule	80	8,395 ± 145 (ref. 36)	11,600
773	Algal nodule	68	8,300 ± 135 (ref. 36)	11,000
			8,600 ± 080	
748	Neomorphic l.st (Original calcite is replaced, so younger ages)	60*	7,845 ± 130 (ref. 36)	10,000
756	—	90	9,435 ± 145 (ref. 36)	12,000
765	—	97	8,380 ± 140 (ref. 36)	12,900

*These points are not plotted in Figure 1.

Several samples (about 90 m depth) on the carbonate platform yielded ages of about 9,000 years BP. Contrastingly, the shells dated 9,135 years BP were collected at 58 m depth on the continental shelf, south of the carbonate platform (see Figure 2). Therefore, there is no particular trend in the data when viewed the ages of unconsolidated sediments and rocks together (Figure 2). The ages on the platform are younger than those at corresponding depths on the eustatic sea-level curve (Table 1) and with the data on the east coast of India. For example, the platform lies between 80 and 90 m depth and the sample ages range from 10,400 to 8,340 years BP. The eustatic sea-level for the corresponding ages was, however, at depths between 65 and 30 m, respectively⁵. Similarly, the ages of the terraces at 80 and 100 m water depth on the eastern shelf of India are 10,790 and 12,530 years BP⁷. The samples corresponding to these ages, however, occur at about 130–150 m water depth on the western margin (Table 1). These anomalies need to be explained based on the nature of sediments.

Constituents and topographic features. Several workers^{8–12} referred to the carbonate (aragonitic) sands and limestones on the platform as oolites and oolitic limestones. If these sands are oolites (chemical precipitates), formed during the Holocene transgression, one would expect older ages seaward and younger ages landward of the platform. This, however, is not evident in Figure 1.

There are several dates on the platform showing an age 10,000–9,000 years BP during which the eustatic sea-level rise was very rapid (23 m/1000 years)⁵. As the formation of oolitic coating is a slow chemical process¹³, it may be difficult to achieve prerequisite conditions (supersaturated aragonite in the waters and maintaining < 10 m depth) for oolite formation at this high rate of sea-level rise. Even if oolites are present at places (possibly associated with terraces or thin oolitic coating formed after 9,000 years BP), large nucleus material determines the radiocarbon ages and one has to find a suitable explanation for the origin of large homogeneous aragonitic nucleus that formed prior to the oolitic coating; this explanation, however, is lacking from the above references. So, referring to the carbonate sands on the platform as oolites does not explain the anomalous distribution of radiocarbon dates (Figure 2). Even if oolitic sediments occur locally, their ages should not be used for the construction of sea-level curve as oolites are unreliable sea-level indicators¹⁴.

A recent study¹⁵ based on bathymetry, seismics and side scan sonar data and on petrology of the sediments and sedimentary rocks has demonstrated the presence of Late Quaternary *Halimeda* bioherms (Figure 4) on the Fifty Fathom Flat. *Halimeda* (Figure 5A) contributed aragonite sediments to the platform. The following points have emerged^{15,16}: (i) High energy environments prevailed during the *Halimeda* growth. (ii) The aragonite muds derived from *Halimeda* were fixed in the form of faecal

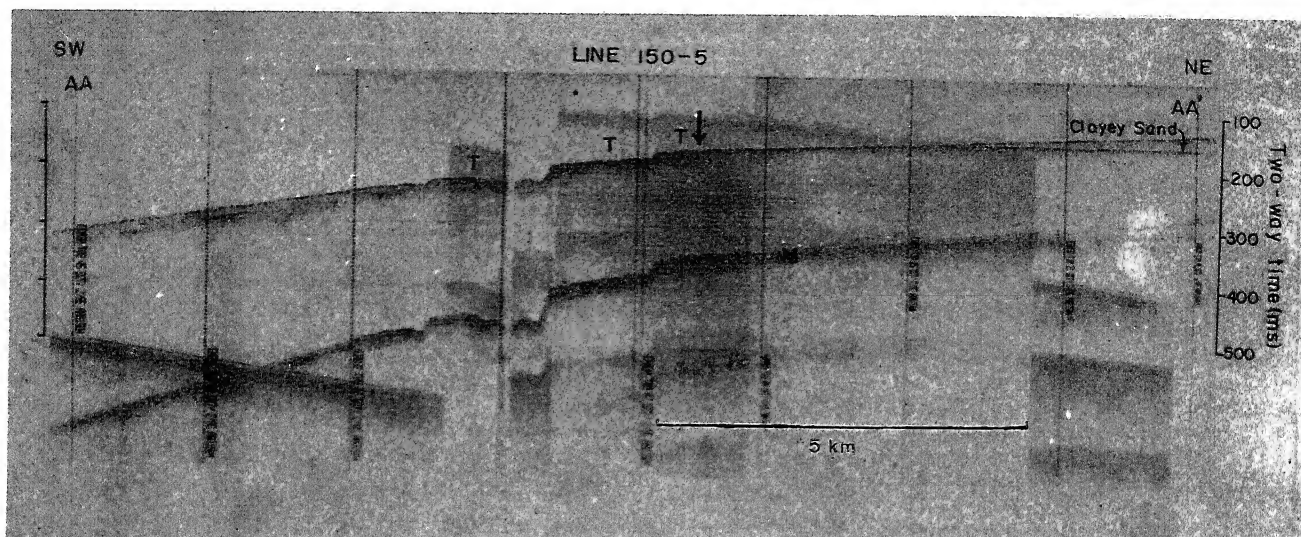


Figure 3. Deeper submarine terraces on the continental slope off Saurashtra-Bombay (see Figure 2 for location). T, terraces; M, multiple.

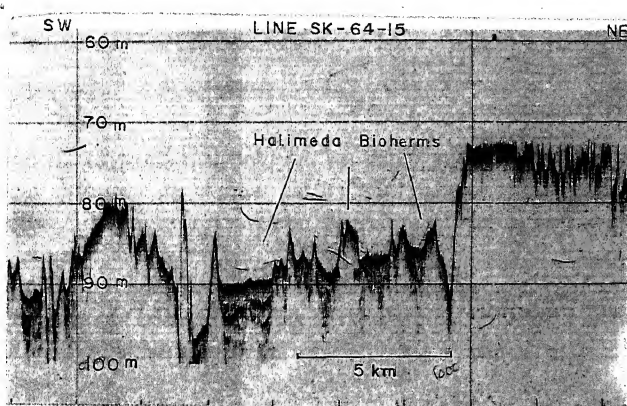


Figure 4. *Halimeda* bioherms on the Fifty Fathom Flat (see Figure 2 for location).

pellet sand (Figure 5B) mainly by Crustaceans. (iii) Reworking of aragonitic sands in high energy environments led to the exposure of older sediments at certain depths (see Figure 2). (iv) *Halimeda* growth continued locally at places until 8,300 years BP, resulting *Halimeda* limestones having younger ages at seaward edge of the platform (see Figure 2). (v) The limestones dated 9,200 years collected at 85 m depth on the platform exhibit vadose diagenetic conditions.

Well-defined submarine sand ridges have also been reported near the shelf break and on the carbonate platform where *Halimeda* bioherms profusely occur¹⁷. These ridges are 1.5 to 18 m high, 0.5 to 10 km wide, several tens of km long, a few kilometers apart and trend almost parallel to the shelf edge (Figure 6). Some broad elongated ridges are superimposed by sand waves/bed forms having heights in the order of 1–2 m

and wavelength 50–300 m. Wagle and Veerayya¹⁷ suggested that these are relict nearshore sand ridges formed in high energy environments where water depths were shallower and tidal and wave-induced currents were stronger.

Since the material that formed sand ridges is aragonite sands which comprise of abundant *Halimeda* fragments and faecal pellets, it is obvious that the *Halimeda* growth on the platform might be prior to and had continued during the sand ridges formation. Both sand ridges and *Halimeda* bioherms indicate high energy environments and the former are distinct towards the edge of the shelf (Figure 6). It is difficult to assess the depth limits for *Halimeda* bioherms, because *Halimeda* normally grew at much shallow depths (< 20 m). However, their growth at depths > 50 m towards the edge of the shelf¹⁸ and certain species of *Halimeda* at 120–150 m depths¹⁹ have also been reported. Reworking exposed older sediments at places. Therefore, the radiocarbon ages of the unconsolidated sediments from the platform may not be of much use in reporting sea-level changes. It has been suggested that all shallow water deposits are not necessarily sea-level indicators and caution be exercised in selecting the sea-level indicators^{1,2}. The depth precision of the sea-level indicators should be well defined before being used for sea-level changes.

Diagenetic textures of the limestones. *Halimeda* limestones dated 8,465 years BP occur towards shelf edge and exhibit marine cements¹⁵. These were similar to subtidal limestones²⁰, wherein pumping of sea water into the pores of the sediments results in cementation. The limestones at 85 m water depth exhibit vadose diagenetic textures, suggesting vadose conditions on the platform at about 9,200 years BP (refs 15, 16). The mineralogy

and stable isotopes¹⁵ suggest that these samples are reliable sea-level indicators. However, the eustatic sea-level was at -40 m at about 9,200 years BP. The differences in the depths thus suggest subsidence of the platform. In fact, the deeper submarine terraces (evidence 1) lie seaward of the platform (see Figure 2) and the samples both from terraces and platform support neotectonic activity. It is not known whether the neotectonic activity after 12,000 years was single or episodic. Sampling on deeper terraces from the slope and other recently described shelf terraces²¹ may yield better information.

Onshore data

There are no samples dated <8,000 years BP, yet available from the offshore. The youngest age so far reported from the calcareous deposits on the outer shelf (Figure 2) and peat/wood from the inner shelf (discussed

below) is 8,300 years BP. It is still unknown the position of the sea-level at 8,300 years BP from the offshore data. On the other hand, Patel *et al.*²² (cf. Merh²³) reported the Early Holocene high sea-level (+8 to +10 m above the present sea-level) in coastal Gujarat and Maharashtra that gradually regressed to the present position at about 6,000 years BP. Sukhtankar²⁴ reported Holocene marine terraces at 4 m above the present sea-level along Maharashtra coast. They could not indicate the exact time of high sea-level during the Early Holocene. Bruckner²⁵ identified marshy soils dated 7,600 years BP, off Gujarat, lying 1.5 m above the sea-level. Juyal *et al.*²⁶ reported oyster reefs at 1-3 m elevation whose ages range from 2,000 to 8,000 years BP, suggesting high sea-level during Early Holocene on the Saurashtra coast. The absence of data <8,000 years BP, from the offshore and the presence of corresponding (Early Holocene dated samples) data in the coastal region imply that the sea-level was already at and above present sea-level by 8,000 years BP. The eustatic sea-level was, however, at -24 m at 8,000 years BP (ref. 5). These differences in depths again indicate possible neotectonism at about this time.

Authigenic clays

Verdine and glaucony are two authigenic green clay mineral facies occurring in tropical shallow marine environments. Verdine occurs at depths < 60 m and glaucony occurs seaward of verdine at depths²⁷ > 60 m. Phyllite V and Phyllite C are the mineral phases of the verdine facies. As verdine is susceptible for alteration, it gets destroyed easily in older surficial marine sediments and therefore it has been found so far to be only of Late Quaternary age²⁷. The depth distribution of verdine and glaucony facies has been verified and used for the reconstruction of palaeogeography of the continental margins during the Late Quaternary²⁷.

The distribution of green grains on the Kerala continental shelf and slope (Figure 7) shows that verdine occurs at depths between 40 and 280 m followed by glaucony down to 420 m depth²⁸. It has been suggested that the verdine and glaucony facies on the continental

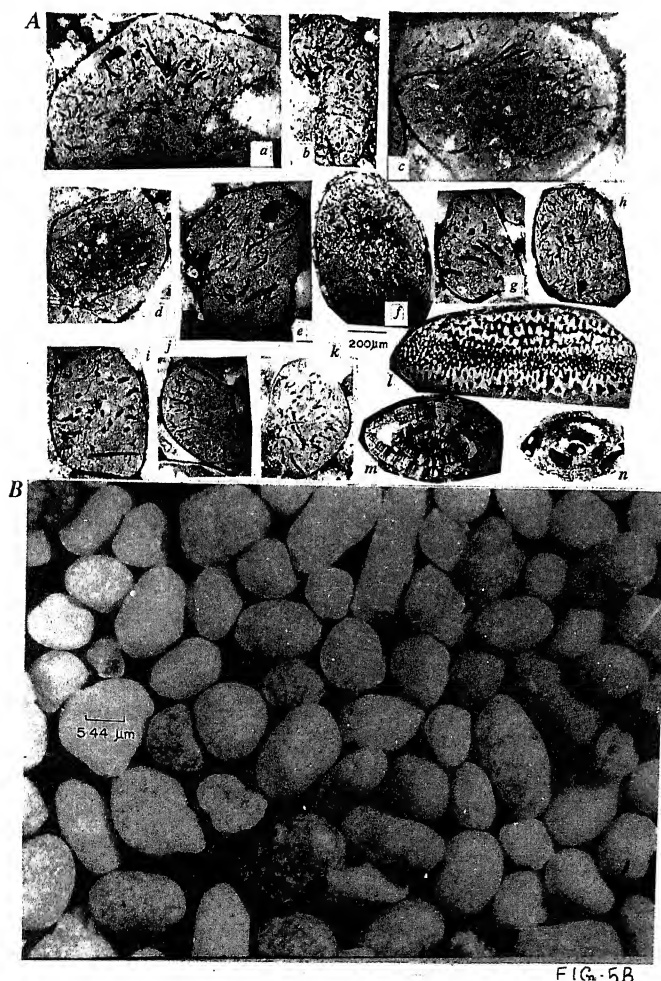


Figure 5. A, Sections of *Halimeda* (a-k), echinoderm (l) and benthic foraminifers (m & n) from the limestones of the platform. Note rounding of *Halimeda* grains. *Halimeda* is aragonitic in composition. If sections are not made, the morphology of the grains can easily be confused with the faecal pellets or oolites. B, Faecal pellet-dominated aragonite sands from the platform.

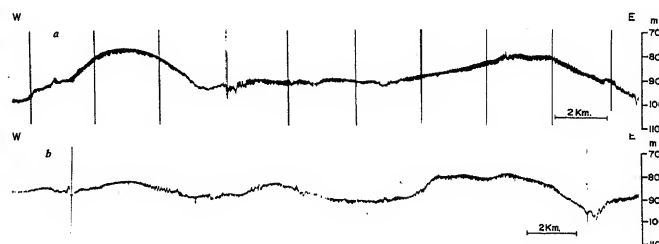


Figure 6. Sand ridges on the continental shelf off Bombay (see Figure 2 for location).

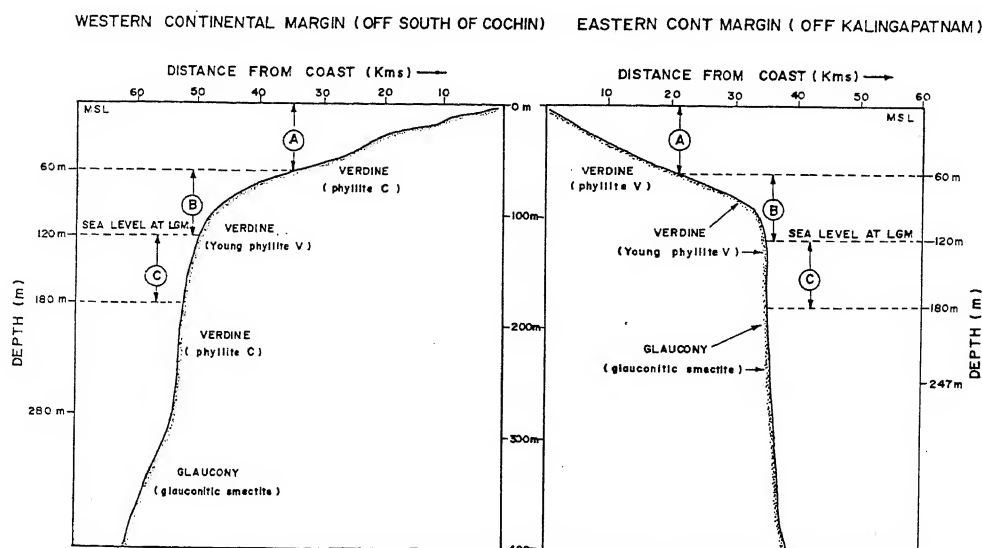


Figure 7. Distribution of green grains on the eastern and western continental margin of India. Zone A is the depth range (0–60 m) for the formation of Present day verdine, zone B is the depth range (61–120 m) for verdine formed during Holocene transgression and zone C (120–180 m) is the depth range for verdine formed during the Last Glacial Maximum.

margin are diachronous and slope facies formed during LGM, when the sea-level was at –120 m, and shelf facies formed during the Holocene transgression²⁷. So the expected depth ranges for verdine and glaucony facies on the slope during the LGM are 120 to 180 (120 + 60) m and > 180 m, respectively. However, on the continental slope of Kerala verdine occurs down to a depth of 280 m. Verdine mineral at 100–205 m (phyllite V) and at 280 m (phyllite C) are different and rules out the possibility of lateral transport. So the distribution of verdine and glaucony facies on the continental margin off Kerala does not coincide with the suggested depth range. On the other hand, verdine facies occur at depths between 18 and 170 m followed by glaucony down to 247 m on the east coast of India²⁹. The range of green grain facies from verdine to glaucony at 170 m depth on the continental slope of the east coast of India (Figure 7) is well in agreement with the suggested depth ranges of these facies during the LGM, and also their distribution is similar to that on Senegalese and French Guinea margins²⁷. The distribution of verdine and glaucony on the west coast therefore appears anomalous and suggests that the paleogeography of the west coast was different during the Late Quaternary, pointing to the possible neotectonic activity. As these authigenic clays form in wide depth range, quantitative aspect of sea-level changes cannot be deciphered from them.

Reassessment of offshore data and sea-level curves

1. There is a paucity of data on sea-level changes for the periods between 18,000 and 12,000 years BP and

8,300 years BP to Present from the offshore region.

2. Although one should appreciate the efforts of earlier workers for attempting sea-level curves for the western margin of India with limited data, these curves may not work as they^{3,4} have used oolite ages and some estimated and inferred ages based on the presumption of oolites which are unreliable sea-level indicators¹⁴. They⁴ have also used radiocarbon ages (range from 9,630 to 8,300 years BP) of carbonized wood/peat^{30,31} from the inner shelf clays. (i) The peat dates are higher as it has been established that mangroves started growing on the tropical shelves world wide during Mid-Holocene and the Holocene peats are nowhere older than 7,000–5,000 years BP (ref. 32). (ii) It has been observed that the age of the peat layer from a core collected at 29 m depth is 9,630 years BP and another peat layer from a core collected deeper offshore at 40 m depth is 8,620 years BP (ref. 31). These dates should have been exactly opposite if peat is sedentary (a sea-level indicator) and if one is to trace the course of Holocene transgression on the western shelf. (iii) The peat ages on the inner shelf and ages of the carbonate deposits on the outer shelf (see Figure 2) are similar suggesting that one of them is allochthonous. The carbonate deposits are extensive on the platform, derived from the *Halimeda* bioherms and thus were not transported. The above points suggest that the peat in the inner shelf clays is most probably allochthonous similar to the peat reported by Mascarenhas *et al.*³³ in the inner shelf sediments off Karnataka and their ages thus do not qualify for the construction of sea-level curve.

3. There are several radiocarbon dates on algal limestones^{8,9,34–36} (Table 1). Unless the specific algae-forming

limestones are identified³⁷, these limestones alone cannot be considered as reliable sea-level indicators³⁸.

4. Since the diagenetic textures have been investigated for the limestones having ages between 12,000 and 9,000 years BP, this part of the curve is constructed and shown in Figure 1. A comparison of this with eustatic sea-level curve of Fairbanks⁵ reveals about 40 m subsidence on this margin sometime after 12,000 years BP. The rate of subsidence and timing of the Holocene neotectonic activity are yet to be established.

5. Practically little radiocarbon ages data exist for the SW continental margin of India.

Despite there were two international efforts (IGCP Projects 200 and 274) for obtaining sea-level data, the Indian continental margins have received very little attention and the construction of sea-level curve for this region is still at its infancy. Sea-level data is of crucial importance, because it provides knowledge on the past sea-level stands based on which we can accurately model and estimate the impact of sea-level rise as a consequence of global warming. More serious and concerted efforts by geomorphologists, sedimentologists and radiochemists are required and would be useful in obtaining this important information.

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Aerodynamic performance of SUNYA and OSHO airfoils

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The inverse design problem of an airfoil where one gets an airfoil for given velocity distribution has engaged the best brains. In revisiting this problem we discovered two new airfoils called SUNYA and OSHO. Aerodynamic performance of these airfoils is given using the existing codes.

In 1977 we gave an analytical airfoil representation¹ describing a symmetrical airfoil in terms of Wagner functions. This representation is given by

$$y' = f'(x) = \sum_{n=0}^{\infty} a_n h_n(x) - a_0, \quad (1)$$

where $f(x)$ describes the airfoil thickness distribution and dash denotes the differentiation w.r.t. x ; $h_n(x)$ are Wagner functions defined as follows:

$$h_n(x) = \frac{1}{\pi} \frac{T_{n+1}(1-2x) + T_n(1-2x)}{\sqrt{x-x^2}}, \quad (2)$$

where $T_n(1-2x)$ is the n th Chebychev polynomial. Properties of Wagner functions are given elsewhere². Figure 1 gives the airfoil geometry and Figure 2 gives the first five Wagner functions. It should however be noted that this expansion need not be a universal one.

Integrating (1) and ensuring the closure of the airfoil at the leading and trailing edges one gets the following representation of the airfoil:

$$f(\theta) = \frac{a_0}{\pi} (\theta + \sin \theta) - a_0 \sin^2 \frac{\theta}{2} + \frac{1}{\pi} \sum_{n=1}^{\infty} \left(\frac{\sin n + 1\theta}{n+1} + \frac{\sin n\theta}{n} \right) a_n, \quad (3)$$

where

$$x = \sin^2 (\theta/2). \quad (4)$$

At that time (1977), we had not realized that this representation has many important consequences for the airfoil design. Recently we³ showed that this expansion contains two new symmetrical airfoils called SUNYA and OSHO as follows.

$$y_{\text{sunya}} = \frac{a_0}{\pi} [2 \sin^{-1} \sqrt{x} + 2\sqrt{x(1-x)} - \pi x], \quad (5)$$

$$y_{\text{osho}} = \frac{a_0}{\pi} [2 \sin^{-1} \sqrt{x} + 2\sqrt{x(1-x)} - \pi x] + \frac{a_1}{\pi} \sqrt{x} (1-x)^{3/2}, \quad (6)$$

where a_0 and a_1 are constants describing the properties of these airfoils. It can be easily verified that OSHO airfoil is the superposition of SUNYA and JOUKOWSKI

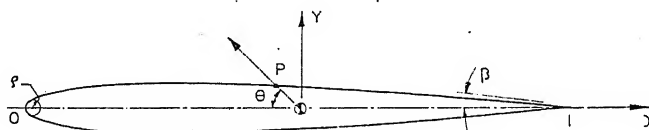


Figure 1. Aerofoil geometry.

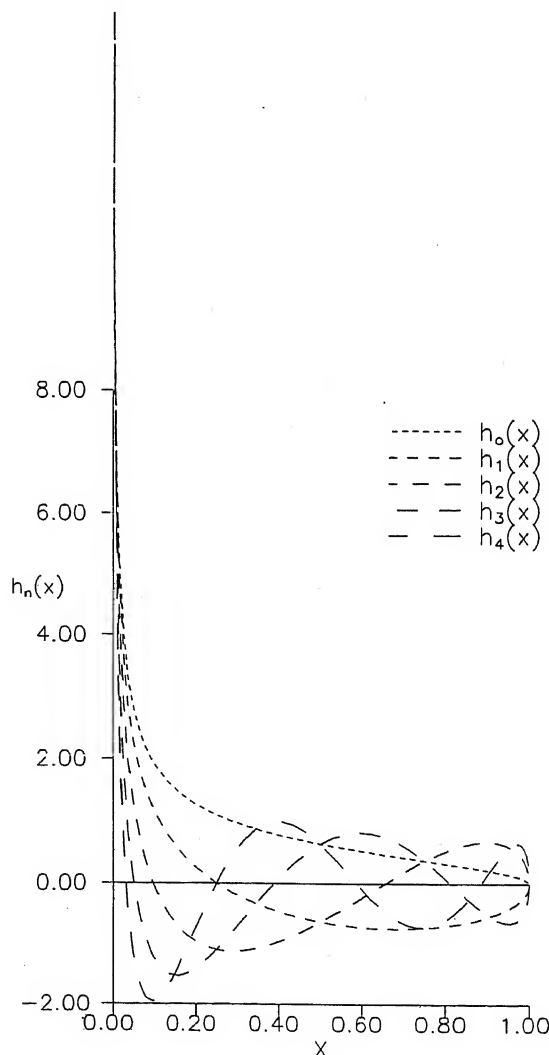


Figure 2. Wagner functions $h_n(x)$ for $n=0$ to $n=4$.

airfoils. It is interesting to compare these airfoils with other classical analytical airfoils given below

$$y_{\text{joukowski}} = \frac{4a_0}{\pi} \sqrt{x} (1-x)^{3/2}, \quad (7)$$

$$y_{\text{kar.trefftz}} = a_0(1-x)^{3/2} \sqrt{x} + a_1(1-x)x, \quad (8)$$

$$y_{\text{piercy}} = a_0 \sqrt{x} (1-x)[1 + a_1(1-x)]^{1/2}. \quad (9)$$

It is recalled that the three latter airfoils are obtained by conformal transformation methods.

In reference 3 we have shown how to use SUNYA and OSHO airfoils for analysis and design of any arbitrary symmetrical airfoil. The properties of these airfoils are given below.

Properties of SUNYA airfoil

- SUNYA airfoil is described by the equation (5) and is a single parameter airfoil, the parameter being a_0 .

The leading edge radius ρ_s is given by the equation

$$\sqrt{\frac{\rho_s}{2}} = 2 \frac{a_0}{\pi}. \quad (10)$$

The trailing edge angle β (positive with respect to negative x-axis) is given by the equation

$$\tan \beta = -a_0, \quad a_0 > 0. \quad (11)$$

- The maximum thickness position x_{max} is given by

$$x_{\text{max}} = \sin^2(\theta_m/2) = 0.2844,$$

$$\text{where } \theta_m = 2 \tan^{-1}(2/\pi). \quad (12)$$

- The maximum thickness is given by

$$t_{\text{max}} = 4(a_0/\pi) \tan^{-1}(2/\pi). \quad (13)$$

All the symbols and variables are defined in Figure 1.

Properties of OSHO airfoil

- OSHO airfoil is described by the equation (6).

Its leading edge radius ρ_{osho} is given by

$$\sqrt{\frac{\rho_{\text{osho}}}{2}} = \frac{2}{\pi} [a_0 + a_1]. \quad (14)$$

- The trailing edge angle β is given by the equation

$$\tan \beta = -a_0, \quad a_0 > 0. \quad (15)$$

- Maximum thickness position x_{max} is given by

$$x_{\text{max}} = \sin^2(\theta_m/2),$$

where

$$\frac{[2(1 + \cos \theta_m) - \pi \sin \theta_m]}{[\cos \theta_m + \cos 2\theta_m]} = -\frac{2a_1}{a_0}. \quad (16)$$

- The maximum thickness is given by

$$\begin{aligned} \frac{t_{\text{max}}}{2} &= \frac{a_0}{\pi} [\theta_m + \sin \theta_m] - a_0 \sin^2 \frac{\theta_m}{2} \\ &+ \frac{a_1}{\pi} [\sin \theta_m + 0.5 \sin 2\theta_m]. \end{aligned} \quad (17)$$

Table 1. 15% SUNYA, OSHO and JOUKOWSKI airfoil coordinates

X/C	SUNYA	OSHO	JOUK
0.00000	0.00000	0.00000	0.00000
0.00099	0.00810	0.00809	0.00724
0.00394	0.01578	0.01575	0.01442
0.00886	0.02302	0.02299	0.02145
0.01571	0.02981	0.02978	0.02827
0.02447	0.03614	0.03611	0.03481
0.04759	0.04737	0.04736	0.04683
0.06185	0.05226	0.05227	0.05219
0.09549	0.06060	0.06063	0.06139
0.11474	0.06404	0.06407	0.06516
0.13552	0.06699	0.06704	0.06833
0.15773	0.06947	0.06952	0.07090
0.18129	0.07148	0.07153	0.07284
0.20611	0.07303	0.07307	0.07416
0.23209	0.07412	0.07415	0.07487
0.25912	0.07477	0.07479	0.07497
0.28711	0.07500	0.07500	0.07448
0.31594	0.07482	0.07480	0.07344
0.34549	0.07424	0.07419	0.07188
0.37566	0.07329	0.07322	0.06983
0.40631	0.07198	0.07188	0.06734
0.43733	0.07034	0.07021	0.06446
0.46860	0.06839	0.06823	0.06124
0.50000	0.06615	0.06596	0.05744
0.53140	0.06365	0.06343	0.05400
0.56267	0.06092	0.06067	0.05010
0.59369	0.05797	0.05770	0.04609
0.62434	0.05485	0.05456	0.04201
0.65451	0.05158	0.05127	0.03794
0.68406	0.04819	0.04786	0.03392
0.71289	0.04471	0.04436	0.03000
0.74088	0.04116	0.04081	0.02622
0.79389	0.03400	0.03366	0.01925
0.81871	0.03045	0.03012	0.01613
0.84227	0.02697	0.02665	0.01328
0.86448	0.02357	0.02327	0.01071
0.88526	0.02029	0.02001	0.00845
0.90451	0.01716	0.01691	0.00648
0.93815	0.01147	0.01128	0.00344
0.95241	0.00896	0.00880	0.00234
0.96489	0.00671	0.00659	0.00149
0.97553	0.00475	0.00465	0.00087
0.98429	0.00309	0.00303	0.00045
0.99114	0.00177	0.00173	0.00019
0.99606	0.00080	0.00078	0.00006
0.99901	0.00020	0.00020	0.00001
1.00000	0.00000	0.00000	0.00000

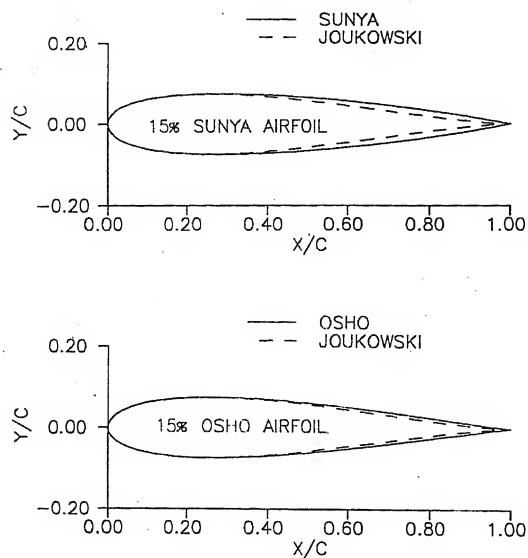


Figure 3. 15% SUNYA and OSHO airfoils.

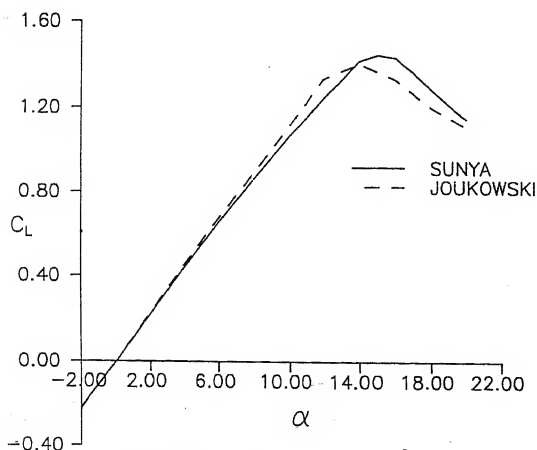
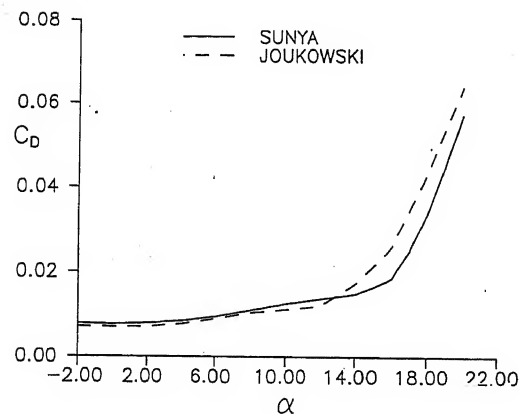


Figure 4. Aerodynamic performance of 15% SUNYA and JOUKOWSKI airfoils ($M=0.1$, $R_e=3 \times 10^6$).

A 15% SUNYA airfoil is obtained by equation (5), where a_0 is obtained by the equation (13) by assuming $t_{\max}=0.15$. Table 1 gives the coordinates of this airfoil. Figure 3 shows this airfoil with 15% JOUKOWSKI airfoil superposed on it. NCSU code⁴ and Rokicki code⁵ are utilized for obtaining the aerodynamic performance at prestall and stalling angles of attack respectively. Figure 4 shows the results at design condition of $M=0.1$ and $R_e=3 \times 10^6$, where M and R_e are Mach number and Reynolds number respectively. The performance of a 15% JOUKOWSKI airfoil is also shown in this figure for comparison.

A 15% OSHO airfoil is obtained by assuming the maximum thickness point to be at $x_{\max}=0.28$ and $t_{\max}=0.15$ in the equations (16) and (17) respectively and evaluating a_0 and a_1 . The coordinates of this airfoil are also given in Table 1. This airfoil is also displayed in Figure 3 with 15% JOUKOWSKI airfoil superposed on it. Again NCSU and Rokicki codes are used for obtaining the aerodynamic performance of this airfoil. Figure 5 gives the results. The performance of the 15%

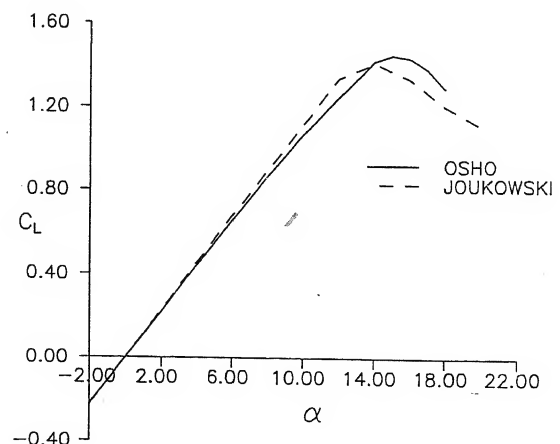
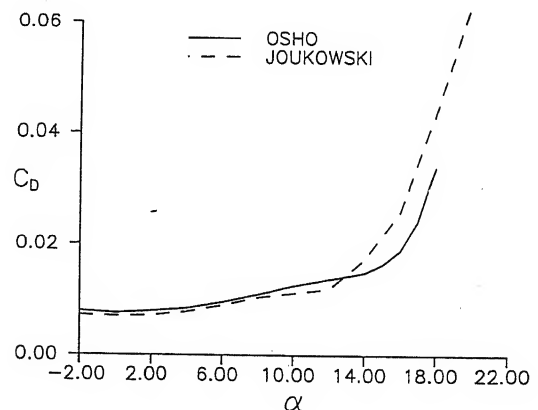


Figure 5. Aerodynamic performance of 15% OSHO and JOUKOWSKI airfoils ($M=0.1$, $R_e=3 \times 10^6$).

JOUKOWSKI airfoil is superposed also on this for comparison.

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Application of equalized molar refraction of zeolites and its correlation with the equalized electronegativity and hardness

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The equalized molar refraction values for various zeolites have been determined by using Sanderson's equalization concept and atomic refraction (R_D) values. Using Komorowski equation, $\eta = (4\pi\epsilon_0 R_D^{1/3})^{-1}$, hardness (η) and equalized electronegativity (χ) are correlated with the equalized molar refraction (R_m). The average deviation was 5%. From the equalized molar refraction various useful properties like molar polarizability, refractive index, etc. can be determined.

THE applications of equalized electronegativity concept to zeolites and other systems are well-established¹⁻⁴. Based on equalized electronegativity, the charges on oxygen and aluminium were correlated to Si/Al ratio of various zeolites⁵. The water content due to the aluminium fraction was also correlated with equalized electronegativity. We have introduced the concept of equalized chemical hardness of zeolites to correlate various physico-chemical properties⁵. Here we report the concept of equalized molar refraction. The equalized molar refraction is correlated with equalized electronegativity and equalized chemical hardness of various zeolites. Hati and Datta^{6,7} have correlated electronegativity/hardness with

electric dipole polarizability of atoms and clusters.

The electronegativity (ψ) and chemical hardness (η) are defined as

$$\psi = (dE/dN)_z = -\mu \quad (1)$$

$$\eta = \frac{1}{2} (d\mu/dN)_z, \quad (2)$$

where E is the energy, N the number of electrons, μ the electronic chemical potential and z the potential due to the fixed nuclei. The equalized electronegativity is represented as,

$$\psi_{eq} = [N/\Sigma (V/\psi)], \quad (3)$$

where N is total number of atoms and V the number of atoms of a particular element or group. The details are given elsewhere⁵.

Similarly $R_{M(eq)}$, equalized molar refraction is defined as

$$R_{M(eq)} = [N/\Sigma (V/R_D)]. \quad (4)$$

$R_{M(eq)}$, the equalized molar refraction is determined⁸ from atomic refractions (R_D).

As given by Komorowski⁹ the chemical hardness can be correlated with ionic (atomic) refractions by the equation,

$$\eta = (4\pi\epsilon_0 R_D^{1/3})^{-1}, \quad (5)$$

where R_D = ionic (atomic) refraction. Based on this Komorowski equation, we have correlated the equalized molar refraction with equalized chemical hardness and equalized electronegativity separately.

The applications of the equalized electronegativity concept and chemical hardness added to our understanding of various materials and provided new method to determine the properties like charges⁵. This prompted us to understand another fundamental property like atomic refraction through equalization concept. Knowing that the chemical hardness is related to ionic (atomic) refraction, we consider the following equation, on the basis of equation (5)

$$\eta_{eq} = A + (B/R_{M(eq)}^{1/3}), \quad (6)$$

where $R_{M(eq)}$ is the equalized molar refraction.

Using the equation (6), 120 zeolites have been studied with the average per cent deviation of 3.72, and the values of the constants A and B are 13.17 and -4.96 respectively. If we consider ionic refraction values instead of atomic refraction values for exchangeable cations, then the average deviation is 5.11% and the values of the constants, A and B are 0.09 and 5.39 respectively.

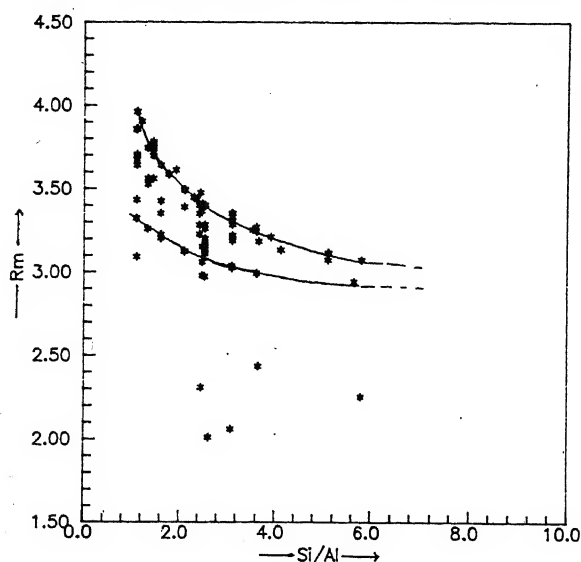


Figure 1. R_M equalized molar refraction vs Si/Al ratio.

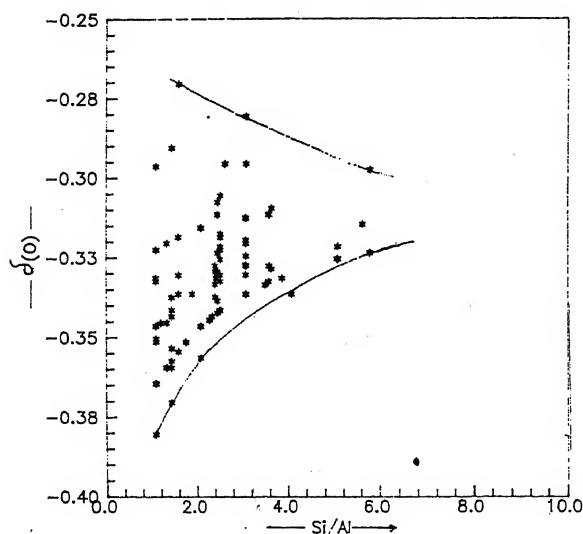


Figure 2. δ_o , charges of oxygen vs Si/Al ratio.

From equations (1) and (2), substituting in eqn. (6), we get

$$\psi_{eq} = A + B \cdot R_{M(eq)}^{2/3} \quad (7)$$

Using equation (7), for 120 zeolites, the average per cent deviation is 2.67 for atomic refraction with $A=11.81$ and $B=-3.12$. While considering ionic refraction values in determining $R_{M(eq)}$, we have obtained the average deviation 4.08% with $A=7.66$ and $B=-0.5349$. Considering the low (6) per cent deviation and the validity of equations (6) and (7), we can determine polarizability (α) or the extent of the electronic

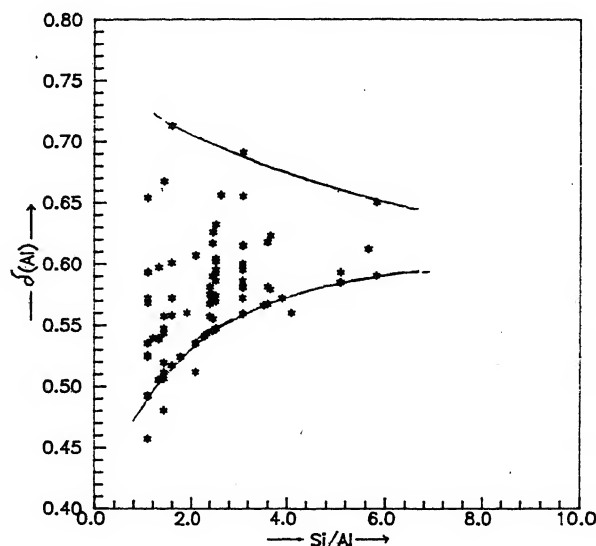


Figure 3. δ_{Al} , charges on aluminium vs Si/Al ratio.

(chemical) polarization in the unit cell. This extent of polarization (α) in turn can be correlated to the trend in the activity of zeolite in the reaction in general and also the coking tendency.

$R_{M(eq)}$, equalized molar refraction of zeolites against Si/Al ratio is given in Figure 1. $R_{M(eq)}$ decreases with the increase of Si/Al ratio. It is interesting to find that $R_{M(eq)}$ remains almost constant for hydrophobic zeolites, i.e. Si/Al ratio >20 (ref. 12). Further, ψ_{eq} values are derived from $R_{M(eq)}$ using equation (7) and using these ψ_{eq} values δ_o and δ_{Al} charges on oxygen and aluminium respectively were determined. The following equation is used to determine the charges,

$$\delta_o = \frac{\psi_{eq} - \psi_o}{\psi_o} \quad (8)$$

δ_o and δ_{Al} with respect to Si/Al ratio are shown in Figures 2 and 3 respectively. The pattern showed that at the same Si/Al ratio, δ varies, probably, as a function of crystallographic structure and nature of cation. On the other hand, we have reported reasonably linear correlation of δ with respect to $(Al/(Al+Si))$, though it also showed some variation with the structure of zeolites⁵. At this stage, it seems that one should be careful in considering the charges from $R_{M(eq)}$ method.

Thus the concept of equalized molar refraction can be considered and there is scope to derive properties like polarizability based on this $R_{M(eq)}$ concept for a wide variety of materials.

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Azadirachtin-induced changes in ecdysteroid titres of *Spodoptera litura* (Fabr.)

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Using radioimmunoassay, insect moult hormone (20-hydroxyecdysone) level was monitored in azadirachtin-injected and control larval (final instar) insects of *Spodoptera litura* (Fabr.). Azadirachtin has significantly reduced the hormone level, which suggests its interference in hormonal regulation, growth and development of the insects.

AZADIRACHTIN, a tetranortriterpenoid, has a number of isomers, of which azadirachtin A contributes the bulk. It has varied effects, viz. antifeedant, repellent, growth regulatory, ovicidal and insecticidal properties against many insects^{1–6}.

Studies on physiological effects of azadirachtin showed that it affects the hormonal titre within the insect through tropic factors. The developmental effects of azadirachtin are attributed to a disruption of endocrine events since the azadirachtin molecule itself has a unique resemblance of the major insect steroid hormone, ecdysone⁷. Complete moult inhibition is shown to be due to either a total blockage of haemolymph ecdysteroid or to a delay in the appearance of the last ecdysteroid peak with or without a reduction in peak height and a slow abnormal decline in the peak^{8–11} or the action of azadirachtin as anti-ecdysteroid by blocking the ecdysone-binding sites^{12,13}. Ecdysone production of prothoracic glands incubated in the presence or absence of azadirachtin and/or prothoracic tropic hormone (PTTH) have shown that azadirachtin does not act directly on the prothoracic glands in *Calliphora vicina*, *Bombyx mori* and *Heliothis virescens*^{14,15}. Further studies showed that azadirachtin blocks the release of neurosecretory material from the corpora cardiaca with a reduced turnover seen as a

subsequent accumulation of material within the system¹⁶. Detailed studies on single injected dose of azadirachtin (1 µg/g body weight) into final instar larvae of *Spodoptera litura* have shown deleterious effect on food utilization, midgut enzymes^{17,18}, haemolymph constituents¹⁹, corpora allata volume, median neurosecretion and carry over effects on ovarian development²⁰.

In the moulting process of insects, ecdysteroids [ecdysone and 20-hydroxyecdysone (ecdysterone)] play a vital role. Ecdysterone titre increased with the age of the larvae. Since the azadirachtin treatment affects the growth and development of the insects, it was interesting to know the effect of the same on those important hormones. In this communication, results on the azadirachtin-induced changes on ecdysterone titres of final instar *S. litura* are discussed.

The haemolymph ecdysteroid titres were determined by RIA basically following Borst and O'Connor²¹, Chang and O'Connor²². Ecdysteroid antibody A and azadirachtin A were gifts from Prof. E. Chang, California and Prof. H. Rembold, Max Planck Institute for Biochemistry, Munchen, Germany, respectively. Radiolabelled ligand [³H]ecdysone (50 µCi mmol⁻¹) was purchased from NEN (USA) and scintillation cocktail (Riatron) was from Kontron (Switzerland).

All other reagents [boric acid, bovine serum albumin (BSA) fraction V, sodium chloride, ammonium sulphate and methanol of extra pure quality] were purchased from SISCO Research Laboratory (India).

Azadirachtin was dissolved in 70% ethanol [(ethanol: water) (70:30 v/v)] and injected (@ 1 µg/g body weight) into the newly moulted VI instar larvae of *S. litura*, at the base of one of the I pair of prolegs, using Hamilton^R syringe. Control larvae were injected with 70% ethanol only. The treatments were replicated five times and all the experimental insects were fed with semi-synthetic diet.

Haemolymph samples (40 µl) were drawn at 24 h interval from azadirachtin-treated and control larvae. Samples (40 µl) were collected in clean sample vials and ecdysteroids extracted with 70% methanol: water (70:30 v/v). The mixture was kept at 4°C for overnight to facilitate precipitation and centrifuged at 2,000 g for 10 min in a table top centrifuge. The supernatant was dried in the counting vials at 36°C.

The schematic diagram for the assay is given in Figure 1. Throughout the assay, RIA buffer [containing borate buffer (100 mM; pH 8.5), BSA (0.1%), sodium azide (0.1%) and sodium chloride (0.5%)] was used, unless otherwise stated. The antiserum was diluted in antibody dilution buffer [same as that of RIA buffer except for the high BSA concentration (5%)]. Radiolabelled ligand was diluted in RIA buffer to get 50% antibody binding and 18,000 dpm radioactivity.

The binding of standard or unknown sample (B) was

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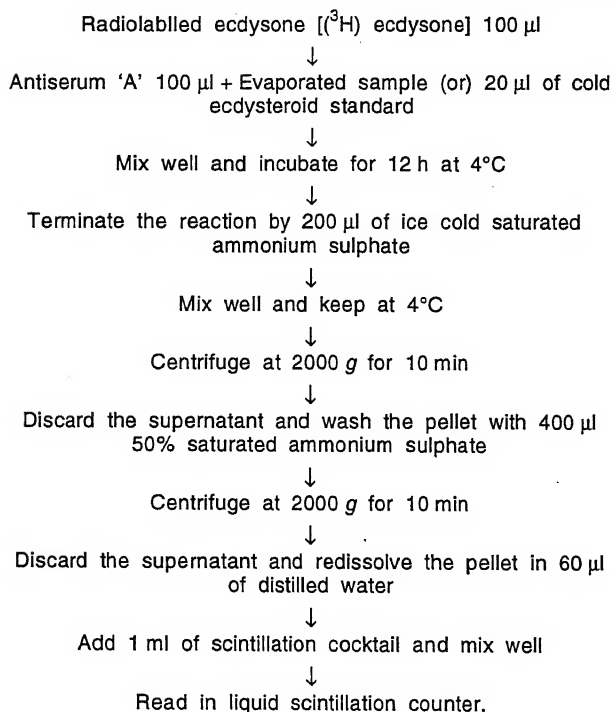


Figure 1. Protocol for the assay.

Table 1. Effect of azadirachtin on 20-ecdysone (ng ml⁻¹) concentration of haemolymph

Hours after treatment	Control	Treatment
24	117.1 ± 5.4	14.7 ± 3.6
48	230.5 ± 14.0	41.2 ± 4.7
72	267.4 ± 19.3	153.7 ± 26.7
96	533.4 ± 43.4	389.9 ± 9.1
120	273.4 ± 12.7	121.7 ± 4.9
Mean	284.5 ± 18.9	144.2 ± 9.8

Comparison by 2-FCRD.

Values are mean (± SE) of five determinations.

CD between main treatment = 16.23.

CD between sub-treatments = 25.67.

CD between main and sub-treatment = 36.28.

expressed as a per cent of maximum binding ($B/B_0 \times 100$). The logit-transformed values were plotted against log-ecdysteroid concentrations (cold ecdysteroid standard – ecdysone and 20-hydroxyecdysone). From the standard curve, the concentration of ecdysterone was estimated by interpolating the values. The titre data were expressed in ng ml⁻¹ 20-hydroxyecdysone equivalents.

The concentration of the hormone at 24 h after moulting into sixth instar was 117.1 ng ml⁻¹ which increased by two folds (230.5 ng ml⁻¹) at 48 h. Azadirachtin treatment dramatically decreased the hormonal level (14.7 ng ml⁻¹) at 24 h. Subsequent increase in the hormonal concentration of the treated larva was similar to that of the normal insects, but with a significantly lower values

(Table 1). Some insects could not complete their life cycle and, both larval (8.7%) and pupal (34%) deformities were observed in the treated insects.

A similar situation was also reported²³ in *S. litura*. The difference between the control and treated in hormonal titres may be due to the effect of azadirachtin on ecdysteroid biosynthesis or its metabolism²⁴.

The haemolymph 20-hydroxyecdysone level remained low during larval feeding and began to increase at the end of the active feeding stage (48 to 72 h) reaching the maximum a day before ecdysis. This is in agreement with the observations on *Plusia aganata*²⁵. It was observed²⁶ that the increase in the haemolymph 20-hydroxyecdysone concentration coincides with the increase in fat body protein level in ligated *S. litura*, suggesting greater synthetic activity of the brain.

The declining hormonal titre in the haemolymph may be due to the greater binding with the peripheral tissues which become physiologically active during this stage, leading to wandering behaviour^{27,28}.

The 20-hydroxyecdysone levels of the haemolymph of the insects reared on semi-synthetic diet are lower compared to those reared on castor leaf²³ which may be due to excessive moisture in the diet causing greater retention by the haemolymph. But the present values are in general agreement with the observations²⁶ on *S. litura* maintained on semi-synthetic diet.

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Isozyme polymorphism in diploid and heat shock-induced tetraploid Indian major carp, *Labeo rohita* (Hamilton)

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Heat shock-induced tetraploids of *Labeo rohita*, an Indian major carp, were studied for esterase, glucose-6-phosphate dehydrogenase (G6PD) and xanthine dehydrogenase polymorphism involving different tissues. Polymorphic loci were identified in eye lens, kidney and skeletal muscle for G6PD and only in kidney for esterase of tetraploids. In general, intensification of isozyme bands was observed in tetraploids. The polymorphism in G6PD-1 and 2 locus in eye lens, G6PD-2 in kidney and G6PD-1 in skeletal muscle and EST-1 locus in kidney could be used as reliable marker in identifying tetraploid stocks from diploids.

ISOZYMES offer a potentially powerful and reliable tool in resolving genetic relatedness/divergence employing the degree of polymorphism of diverse alleles at different

loci involved in translating specific enzymes and their varied multiple molecular forms, i.e. isomers. They have amply been used as molecular tags in genetic, phylogenetic, taxonomic, evolutionary studies and in strain or type identification^{1–5}.

Enzymes are mostly primary products of transcriptionally active genes and it is assumed that specific enzyme profile is the reflection of the genetic make-up of a given species and may be used as 'finger print' considering all other variables as constant. The present study was aimed at identifying any induced variation due to increased ploidy level (diploid to tetraploid) in three different enzyme profiles across diverse tissues and to assessing the feasibility of employing such altered profile (as marker) in identification of tetraploid fishes. Confirmation of tetraploid status has largely been based on chromosome counting⁶, cellular volume measurement⁷, DNA content⁸ and protein electrophoresis⁹.

Ploidy manipulation was found as a novel approach in altering diverse traits in fish system^{8,10} and promising results in many cases encouraged us to undertake the same with *Labeo rohita* in the Central Agricultural Research Institute, Port Blair during 1993. Thermal shock-induced tetraploid stocks¹¹ were developed and reared under in house hatchery and in natural pond condition for evaluation and characterization. Specimens were collected from the polycraft pool and pond after eight months of rearing. After having tetraploid status confirmed (through chromosomal count following standard technique¹²), liver, kidney, eye lens and skeletal muscle were dissected out immediately on sacrificing the experimental fishes. The cell lysate was prepared in tissue homogenizing buffer¹³. Homogenates were loaded and electrophoresed following standard procedure⁵ in a 5% native PAGE using discontinuous buffer system. Isozymes were detected through specific histochemical staining following Shaw and Prasad¹⁴ for G6PD and Paul *et al.*¹⁵ for esterase (EST) and xanthine dehydrogenase¹⁰ (XDH). The mobility of individual band was calculated through the measurement of R_m values.

The profiles of all the three isozymes are presented in Figures 1–3 along with corresponding zymograms. The allelic expression of G6PD (E.C.1.1.1.49) could be distinctly grouped into two regions in liver and three regions in kidney of tetraploids whereas only two activity zones were found in diploids (Figure 1). The anodal band (locus-3) was a homozygous locus and represented by a single intensely stained band in tetraploids with slow mobility. In diploid, this locus was represented by light-stained band coupled with faster mobility. To the contrary, the presence of an additional zone of activity (locus-2) in the kidney was found to be unique in tetraploids. In the eye lens, G6PD was expressed in three distinct loci (locus-1, 2, 3) of tetraploids (Figure 1, lanes 10 & 11). Three alleles of heterozygous nature

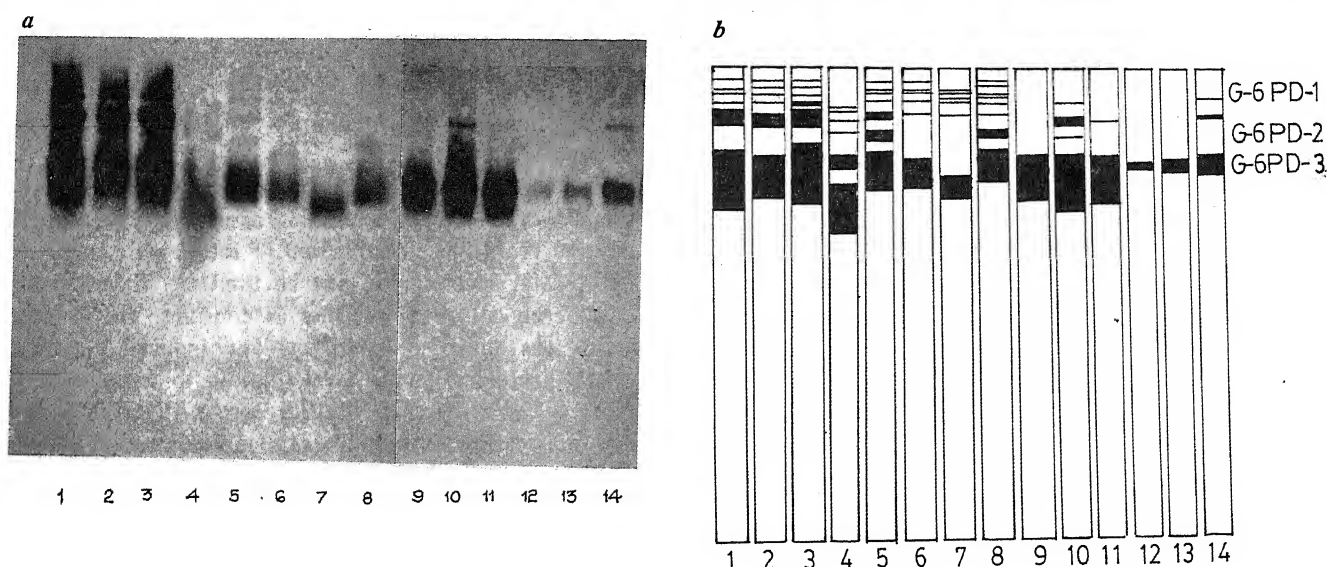


Figure 1. Glucose-6-phosphate dehydrogenase polymorphism in *L. rohita* (diploid and tetraploid). *a*, photograph; *b*, zymogram. Liver: lanes 1–3 (tetraploid), lane 4 (diploid); kidney: lanes 5, 6 & 8 (tetraploid), lane 7 (diploid); eye lens: lanes 10 & 11 (tetraploid), lane 9 (diploid); skeletal muscle: lanes 13 & 14 (tetraploid), lane 12 (diploid).

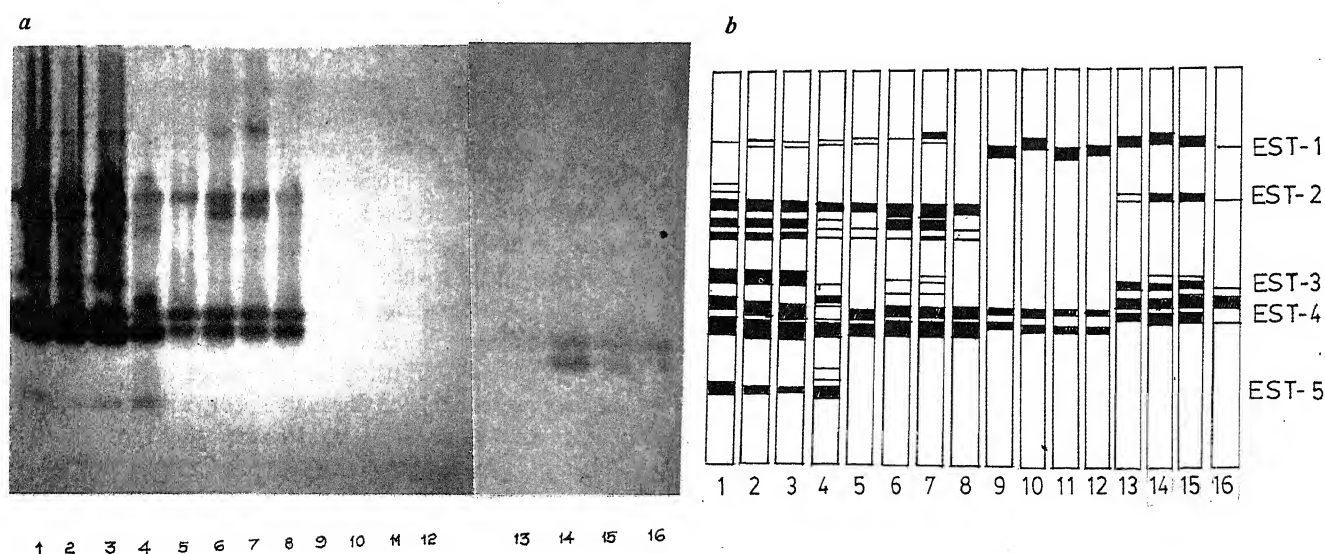


Figure 2. Esterase polymorphism in *L. rohita* (diploid and tetraploid). *a*, photograph; *b*, zymogram. Liver: lanes 1–3 (tetraploid), lane 4 (diploid); kidney: lanes 5–7 (tetraploid), lane 8 (diploid); eye lens: lanes 9–11 (tetraploid), lane 12 (diploid); skeletal muscle: lanes 13–15 (tetraploid), lane 16 (diploid).

characterized by one intensely stained band and two light bands (R_m 0.081–0.1) were found in the cathodic zone. This locus (G6PD-2) was specific to tetraploids. The anodal region, however, in both the diploids and tetraploids was represented by a single intensely stained band with R_m values of 0.298 and 0.317 respectively (Figure 1, lanes 9–11). In skeletal muscle, G6PD was expressed in one locus (locus 3) as a light band encoded by a single homozygous allele in diploids whereas in tetraploids an additional band in locus-1 (R_m values 0.08 and 0.093) having two alleles was expressed.

Information on G6PD polymorphism in fishes is scanty¹⁷. Involvement of one locus in G6PD expression in trout^{18,19} has been reported earlier. Five bands of this isozyme have been observed in the brain and eye tissue of *Heteropneustes fossilis* with identical electrophoretic mobility¹⁶. In the present study, three loci could be identified for G6PD which expressed across different tissues. G6PD activity in the liver, kidney, eye lens and skeletal muscles of *L. rohita* diploids and tetraploids was found variable and distinctly a tissue-specific pattern was observed.

The esterase (E.C.3.1.1.1) activity at different loci in

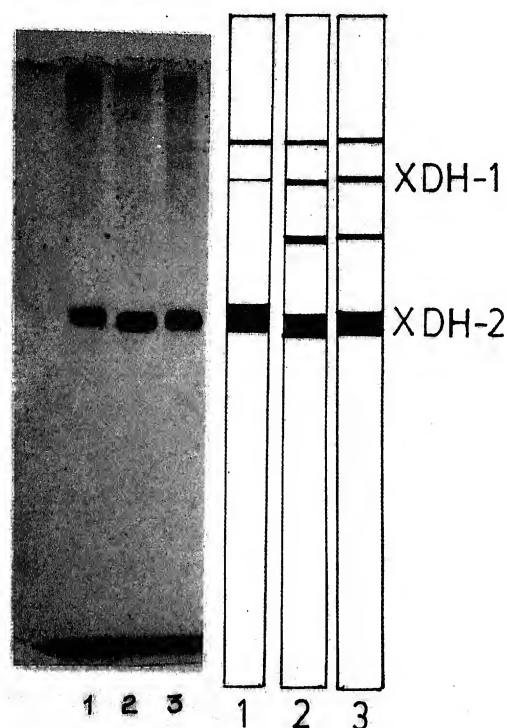


Figure 3. Xanthine dehydrogenase polymorphism in *L. rohita* (diploid and tetraploid). Liver: lanes 2 & 3 (tetraploid), lane 1 (diploid).

liver is well-recognized with diverse R_m values (0.17–0.84), e.g. five zones in liver, four zones in kidney, two zones in eye lens and three zones in skeletal muscles (Figure 2). Unlike diploids, the esterase activity in liver of tetraploids had five zones with intense staining (R_m 0.18, 0.3–0.43, 0.53, 0.60–0.67 and 0.83). Est-1 was found expressed through two alleles in all except in one specimen. In all the tetraploids, Est-3 was represented by three intensely stained bands and Est-4 (R_m value 0.4) was found to be unique for tetraploids coded by a single allele with high enzyme expression. The anodal Est-5 of these specimens were found to be highly active zones compared to diploids (Figure 2, lanes 1, 2 & 3). In kidney, the enzyme activity which was faintly visible at Est-1 and Est-3 loci of diploids (Figure 3, lane 8) was found to be more or less clearly visible zones (still less active regions) in tetraploids, maybe coded by alleles translating less amount of enzyme activity. The isozyme in eye lens of tetraploids and diploids did not show any variation as represented by light bands at Est-1 and Est-5 (Figure 2, lanes 9–12). In the skeletal muscle, esterase activity was expressed in three loci (Est-1, 3 and 5). The allelic expression in tetraploids was characterized with three alleles and one additional less active region (Figure 2, lanes 14 & 15) at the anodal locus (Est-5). Whereas in diploids extremely low activity was observed at Est-1 and 2 (Figure 3, lane 16).

Esterase in all the tissues of tetraploids was found to

express intensely stained bands when compared to the diploids. In locus-3 and locus-5 of liver and kidney, the activity in tetraploids was well marked, being coded by three and two alleles respectively, whereas the activity was very much low for diploids. The presence of highly active Est-4 in liver and Est-1 in muscle could be the characteristic expression of enzyme in tetraploids.

XDH (E.C.1.1.1.204) was expressed at two loci in both diploids and tetraploids. The intense anodal bands of homozygous nature were clearly marked in all individuals (R_m 0.44, 0.45) but the migration of these bands in tetraploids was comparatively faster. The cathodal XDH-1, characterized with light bands (R_m 0.197–0.296), was heterozygous and less expressive in nature. The activity of XDH-1 locus in diploid specimens (lane 1) was almost negligible in comparison to tetraploids (lanes 2 & 3). The phenotypic expression of XDH with a single intensely stained band in liver corroborated the previous observation²⁰ and the presence of three faintly stained bands in tetraploid liver tissue, reported here for the first time, could be used as a marker in identifying tetraploids.

The present study was undertaken to assess the possibility of using altered isoenzyme profile as a genetic marker in identifying tetraploid fishes. G6PD-1 and G6PD-2 of eye lens (R_m values of 0.081 and 0.096), G6PD-1 of skeletal muscle (R_m values 0.08 and 0.94) and G6PD-2 of kidney (R_m value 0.176) were found as distinct marker bands in tetraploids.

In esterase, more intensely stained bands in different loci of tetraploids perhaps indicative of increased gene dose due to gene duplication¹³ and absence of some less active region, might be an epigenetic modification or post-translational modifications. Similar observations were also reported for malate dehydrogenase in herring¹³. XDH in tetraploids was characterized mostly through fast mobility of the intensely stained single band (locus-2).

Appearance of additional bands in tetraploids as against diploids may be assumed due to gene duplication and thereby enhanced enzyme expression in stainable and detectable quantum which probably could have failed to be stained due to negligible amount in diploids. Formation of heteropolymeric isozyme due to hybridization of protein products of separate loci may be another plausible explanation for development of new bands with entirely different R_m values. It is maintainable that hybrid polymorphs have been reported in many fish species and in polyploid organisms that have undergone extensive gene duplication⁵. The chromosomal duplication achieved through ploidy manipulation was well-expressed in manifesting the structurally polymorphic isozymes in fish.

In conclusion, G6PD-1 and 2 in eye lens, G6PD-1 in skeletal muscle, G6PD-2, Est-1 in kidney, Est-4 and

XDH-1 and 2 in liver could be used as reliable marker with ease and confidence in identifying tetraploid stock of *L. rohita*.

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Molecular genetic diversity among soybean plant introductions with resistance to *Heterodera glycines*

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Restriction fragment length polymorphisms were used to estimate the genetic diversity among 29 soybean (*Glycine max*) accessions with resistance to cyst nematode (*Heterodera glycines*). Based on the common

marker alleles, both the analyses cluster and principal component have separated the resistant soybean accessions into several groups. Several accessions with known resistance to some races were also found to be resistant to additional nematode races in this research.

In USA, *Heterodera glycines* Ichinohe (soybean cyst nematode) parasitism is a major limiting factor of soybean (*Glycine max* L. Merr.) production. This nematode was first discovered in USA in 1954 and has since been found in 27 states. In 1994 the estimated soybean yield losses were valued at 115 million dollars for 16 southern states¹.

The management of *H. glycines* primarily relies on the use of resistant cultivars of soybean. Most cultivars are resistant to one or two nematode race isolates and the current classification system includes 16 different race isolates². The modern soybean gene pool for resistance is generally regarded to be genetically very narrow, mainly because the introgression of resistance genes from the available sources is restricted to either cv. Peking or PI88788 or both. The narrow genetic base of the resistance sources used in cultivars has been causing shifts in *H. glycines* populations favouring development of more aggressive races and the resistance has not been durable. Use of non-allelic genes, i.e. genes located at different loci for resistance will produce more durable resistance in soybean cultivars.

Presently, 118 soybean accessions are available, which are resistant to *H. glycines*. Some of them were reported to carry non-allelic genes for resistance^{3,4}, but they have not been utilized in breeding programmes, except for a few resistance genes from PI437654 soybean. This line is resistant to race isolates⁵ 1, 2, 3, 5, 6, 9 and 14. Prior knowledge of the genetic relationships among the accessions would facilitate development of resistance genes to improve genetic diversity and gene pyramiding. Traditional techniques do not offer adequate tools for establishing these relationships.

Restriction fragment length polymorphism (RFLP) markers has been widely accepted for genetic analysis and varietal identification by DNA 'fingerprinting'. Genetic relationship on the basis of single-copy RFLP markers has been reported for several crop species^{6,7}, including soybean⁸. All of these investigations pertain to crop cultivars with known pedigrees.

We are not aware of any report in the literature on the evaluation of genetic relationships among soybean accessions with unknown pedigrees having resistance to *H. glycines*. In the study described here, we surveyed 29 resistant accessions and two susceptible cultivars of soybeans using RFLPs to obtain information on their genetic diversity and relationships. A brief summary of this research has been reported in the *Agronomy Abstracts*⁹.

For this study, seeds from 29 soybean accessions with resistance to *H. glycines* were obtained from R. L.

Nelson, Curator, USDA-ARS, Soybean Germplasm Collection. These resistant accessions represent samples from five countries: Argentina, China, Japan, Russia and South Korea. Two susceptible controls, cv. Essex and cv. Hutcheson were also included in the study.

Near-homogeneous populations of *H. glycines* were developed for each of the five races. 1, 2, 3, 5 and 14 based on the methods described previously¹⁰. Bioassays were performed for each of the five races based on the established procedures³. In brief, each soybean seedling was grown in a single polypropylene micropot (200 × 25 mm) filled with steam pasteurized Brosely fine sandy soil. Approximately 20 of these micropots were placed in a 20 cm diameter polypropylene container and maintained at 27 ± 1°C in thermoregulated waterbaths (Forma Scientific Inc., Marietta, OH, USA).

White and light yellow females of a given race of *H. glycines* were chosen selectively and were crushed to release eggs and larvae. Each seedling was inoculated with 1250 ± 25 eggs and larvae using an automatic pipetter (Brewer Automatic Pipetting Machine, Scientific Equipment Products, Baltimore, MD, USA). A total of 40 seedlings were inoculated for each race. The method of inoculation was already described¹¹.

Approximately 30 days after inoculation, plant roots were individually washed with a strong jet of water to dislodge *H. glycines* females and counted under a stereomicroscope. Resistance was determined for each soybean accession and for each of the five races, based on an index of parasitism.

Total genomic DNA was isolated from greenhouse-grown plants of each resistant accession and for each of the two susceptible controls. DNA extracts were obtained according to Keim and Shoemaker¹². Purified DNA was quantified using a Beckman DU-65 spectrophotometer and UV quantitation of DNA method according to Sambrook *et al.*¹³. Samples of genomic DNA were individually digested with restriction endonucleases *Dra*I, *Eco*RI, *Eco*RV, *Hind*III and *Taq*I.

Established procedures were employed for gel electrophoresis, Southern blotting and hybridization with the exception that the hybridizations were conducted using 25–35 ml of hybridization solution at 65°C in glass tubes that were rotating on a rotisserie (Robbins Scientific Corporation, Sunnyvale, CA, USA).

The 32 probes used in the study were primarily single-copy DNA sequences and random clones from a genomic library¹² cloned into the *Pst*I site of the pBS⁺ vector and were transformed into DH5 α strain of *E. coli*. The clones were prepared for radiolabelling by first amplifying the inserts via polymerase chain reaction (PCR) using oligonucleotides of the T₃ and T₇ promoter regions of the phagemid vector pBS⁺ as amplification primers. The amplification was based on the established procedures¹⁴. The PCR amplified inserts were radiola-

belled with ³²P using random priming reactions according to Feinberg and Vogelstein¹⁵.

On a marker basis we have calculated polymorphism index = $1 - \sum p_i^2$, where p is the allele frequency for i alleles 1 to N . On a probe basis, the polymorphic index = $1 - \sum \sum p_{ij}^2$ where different polymorphic loci are summed.

Only the fragments that were polymorphic among accessions and could be clearly scored were used in the data analysis. All bands having equivalent migration distance were given the same letter score. Genetic distances (GD_R) among all possible pairs of accessions were estimated from a modification of Nei's similarity equation¹⁶ as used by Keim *et al.*⁸ in soybean. The proportion of similar RFLP loci, S_{xy} , between pairs of PI lines was estimated as $2N_{xy}/(N_x + N_y)$, where N_{xy} is the number of RFLP loci for which PI lines X and Y possess the same allele, N_x is the number of alleles identified in line X and N_y is the number of alleles identified in line Y. GD_R was calculated as $1 - S_{xy}$. Based on the GD_R matrix (data not presented), a dendrogram was generated to graphically display the calculated distances between genotypes (Figure 1). Cluster diagrams were constructed using the average linkage

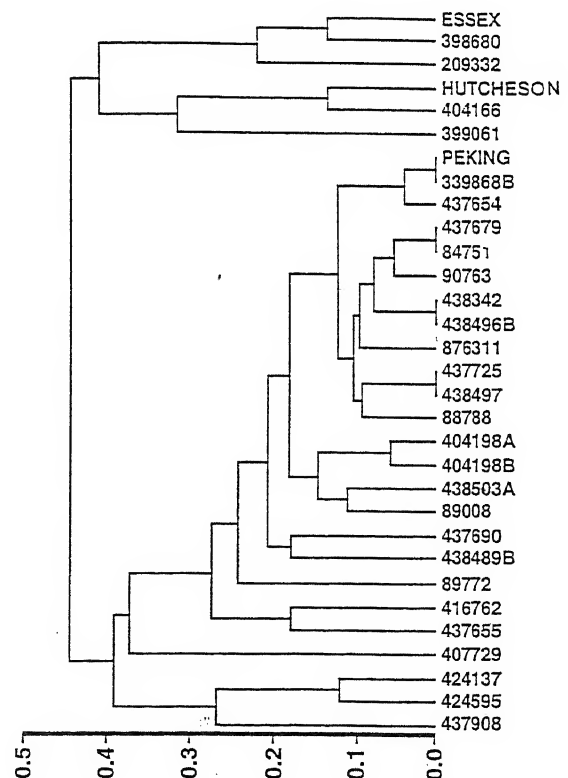


Figure 1. Dendrogram of soybean PIs and cultivars obtained from an analysis of 32 probes. Genetic distances calculated from RFLP values were analysed by average-linkage clustering. The scale on the dendrogram represents the degree of divergence.

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Table 1. Reaction* and index of parasitism** of soybean PI lines to *H. glycines* race isolates 1, 2, 3, 5 and 14

Accession	Race I	Race II	Race III	Race V	Race XIV
PI398680	MS (56.0)	S (78.6)	MR (26.2)	S (67.5)	MS (42.5)
PI209332	MS (47.0)	S (70.0)	R (4.0)	R (7.0)	R (5.0)
PI404166	R (1.0)	R (6.0)	R (0.6)	R (0.4)	MR (10.0)
PI399061	MS (38.0)	MS (41.7)	MR (33.3)	R (7.8)	S (63.7)
Peking	R (1.8)	MR (19.7)	R (1.5)	R (0.9)	MR (28.1)
PI339868B	R (2.0)	MR (20.0)	R (0.8)	R (1.7)	MR (12.6)
PI437654	R (0.2)	R (1.0)	R (0.3)	R (0.4)	R (0.3)
PI437679	MR (22.0)	MR (14.3)	R (4.0)	R (0.1)	R (2.2)
PI84751	R (3.2)	MR (28.0)	R (0.2)	R (1.4)	MR (14.0)
PI90763	R (7.9)	R (2.3)	R (0.4)	R (0.1)	MR (26.8)
PI438342	MS (38.0)	MR (21.6)	MS (41.7)	R (1.7)	MS (32.3)
PI438496B	MR (30.0)	S (96.0)	R (2.3)	MS (35.0)	MS (41.0)
PI876311	MS (38.0)	S (67.3)	R (9.0)	MR (16.6)	MR (10.9)
PI437725	R (0.6)	MR (18.7)	R (0.4)	R (2.4)	MS (45.7)
PI438497	R (5.0)	MR (17.0)	R (0.4)	R (2.2)	MR (29.0)
PI88788	MS (39.0)	S (77.2)	R (5.4)	MS (46.0)	R (2.4)
PI404198A	R (0.7)	R (8.5)	R (1.4)	R (1.0)	MS (54.6)
PI404198B	R (2.0)	MS (44.2)	R (1.7)	R (1.4)	MR (23.6)
PI438503A	MS (44.0)	MS (57.0)	R (2.9)	MR (22.1)	R (9.0)
PI89008	MS (35.0)	S (66.4)	MR (17.9)	MR (17.8)	MR (17.8)
PI437690	R (0.8)	R (7.1)	R (0.0)	R (1.7)	MR (25.0)
PI438489B	R (0.4)	R (4.5)	R (0.6)	R (1.1)	R (8.0)
PI89772	R (1.5)	R (3.5)	R (0.2)	R (0.8)	MR (13.6)
PI416762	MR (16.0)	MS (47.1)	R (6.5)	R (5.7)	R (8.1)
PI437655	R (1.0)	S (69.0)	R (0.6)	R (3.0)	MR (11.5)
PI407729	MR (11.0)	MS (46.5)	MR (14.5)	R (6.3)	R (4.5)
PI424137B	S (61.0)	S (88.0)	MR (34.9)	MR (11.8)	MR (27.5)
PI424595	MS (58.0)	MS (45.2)	S (95.2)	R (9.9)	S (64.9)
PI437908	S (74.0)	S (80.9)	S (95.2)	MS (54.8)	S (84.7)
cv. Hutcheson	S (100)	S (100)	S (100)	MS (100)	S (100)

*0-9% = Resistant; 10-30% = moderately resistant; 31-60% = moderately susceptible; ≥ 60% = susceptible.

**Index of parasitism = $\frac{\text{Average number of females per PI line}}{\text{Average number of females per cv. Hutcheson}} \times 100$.

cluster analysis (Statistical Analysis Systems, Cary, NC) on the distance matrices.

The principal component analysis (PCA) was done by first calculating a correlation matrix among the markers. Eigenvalues and eigenvectors were then obtained from the correlation matrix and these were used to calculate the coordinates of each accession. The accessions were then plotted on the basis of these coordinates (data not presented).

The reaction of PI lines to races of *H. glycines* is reported in Table 1. Results indicate that several PI lines with known resistance to some races were also found to be resistant to additional nematode races (Table 1). For example, PI438489B was resistant to races 3 and 5 and in this study it was found additionally resistant to *H. glycines* races 1, 2 and 14. Of the 32 probes examined, 13 were polymorphic producing two to nine restriction fragment bands. We found that 40% of the probes detected variation among the 29 resistant and two susceptible soybean genotypes.

Both cluster and principal component analyses separated the accessions and cultivars into several groups. Cluster analysis has grouped genotypes based on the

proportion of marker alleles that accessions and cultivars have in common. Genotypes that have a high proportion of alleles in common included 'Peking' and PI339868B; PI437679 and PI84751; PI438342 and PI438496B; and PI437725 and PI438497 (Figure 1). These pairs are distinguishable but very closely related based on the RFLP data.

Based on both the cluster analysis and the principal component analysis (data not shown) most widely used genetic sources for *H. glycines* resistance have 'tight' grouping or clustering. These included Peking, PI88788, PI90763, and PI437654. A few of the resistance genes in both the accessions PI437654 and Peking have been recently mapped using RFLPs^{17,18}. Genetic analyses have indicated that very few non-allelic genes exist among these sources of resistance¹⁹, and their indiscriminate use in cultivar breeding appeared to have contributed to their genetic vulnerability. More virulent race populations of *H. glycines* are being reproduced which continue to infest the resistant cultivars.

Some PI lines with multiple race resistance that were genetically distant from the previously used sources were identified in this study (Table 1). These included

PI438489B, PI404198B, PI438503A, PI89772, PI404166, PI437908, PI209332 and PI437655. The resistance genes in these PIs will be mapped.

The results showed that greater diversity for markers existed among the PIs than the two cultivars used in this study. The two cultivars Essex and Hutcheson were also clustered away from the PIs in the dendrogram (Figure 1). This is expected, because the PIs are in general a diverse group of accessions collected in Asia and South America, whereas the cultivars were derived from a limited number of PIs introduced in USA at the turn of the century. In this study, no relationship was found in general, between geographical origin of PIs and the clusters obtained based on the limited number of probes used, but some degree of relationship was observed for spectrum of resistance. Both PI424595 and PI437908 susceptible to races 3 and 14 which clustered at the lower end of dendrogram have originated from South Korea.

Our study has demonstrated that an analysis of RFLP markers can be used to determine genetic relationships among PI lines of soybean. In the absence of typical pedigree information, DNA fingerprinting should be most useful in establishing their genetic relationship to develop appropriate populations for gene mapping studies.

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Aeolian deposition of Arabia and Somalia sediments on the southwestern continental margin of India

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Kaolinite, smectite, illite and chlorite as major clay minerals and palygorskite and gibbsite in minor quantities have been recorded from the slope of southwestern continental margin of India. Contribution of kaolinite, smectite and gibbsite is from peninsular India through fluvial discharge. Since formation of palygorskite calls for an arid and hot climate and saline conditions, occurrence of this clay mineral in the sediments of the study area documents aeolian sediment contribution from Arabia and Somalia by the Arabian northwesterly winds.

STUDIES of marine clays are a significant tool to determine sources, sediment dynamics and environment of deposition¹⁻⁴. The climate and geology of the source area³ largely dictates the type of clay species supplied. By and large, characteristic clay minerals of different climatic and geological settings have been identified^{1,3}.

Studies of clay mineral variations in the western continental margin of India mainly suggest two important sources of the clays. Illite and chlorite are reported to be mostly contributed by the Indus River and the low salinity Bay of Bengal Waters (BBW), intruding into the southwestern continental margin during November-January⁵⁻¹¹. Kaolinite, gibbsite and smectite (and minor amounts of illite) are produced due to intense chemical weathering of Indian subcontinent, and are contributed from the adjacent landmass⁵⁻⁷. Two contrasting opinions exist about the dispersal of these clay minerals. Ramaswamy and Nair⁹ have reported a lack of cross-shelf sediment transport and have suggested an along shelf transport of the sediments brought by the major fluvial

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routes of the Indian subcontinent. Others^{7,12} proposed across-shelf transport of the sediments and clays and suggested the influence of the climate and the geology of the western continental margin of India on local clay mineral distribution. But, aeolian supply from other continents has not yet been documented.

The present work was carried out between Cape Comorin and Quilon (Figure 1). Clay mineral studies of 51 surface sediment samples are the basis of the present study. Sediment contribution by the aeolian processes to the northwestern and the central Arabian Sea, adjacent to Somalia and Arabia, has been documented⁵⁻¹¹. The present work records sediment contribution by the aeolian process to the southwestern continental margin of India.

During the 158th cruise of *R. V. Gageshani*, 51 surface sediment samples along and across 7 transects, spread between 7.0°–9.56°N and 75.35–78.0°E, were collected using a Peterson grab (Figure 1). The samples were washed to remove salts, oven dried at 70°C and were analysed for texture. Pipette separated, < 2 µm, oriented, and air-dried samples were subjected to X-ray diffraction studies on a Philips diffractometer (model PW 1814) using a nickel filtered CuK_α radiation ($\lambda = 1.54$). Standard glycolation methods¹³ were used to aid the identification of expandable clay minerals. Weighted peak area method¹ was used to quantify the clay species. Palygorskite is identified by slow scanning (rate 0.6 2 θ min⁻¹) between 8 and 20° to resolve its principal peaks at 10.4 Å, 6.36 Å and 4.47 Å, and quantified after the method of Sirocko and Lange¹¹. To check the reliability of analysis, about 20% of samples were reanalysed which revealed $\pm 8\%$ accuracy for smectite and $\pm 5\%$ for other clays.

The major clays observed in the study area are kaolinite, smectite, illite and chlorite in the order of abundance (Figure 2). Others present in minor amounts are gibbsite (~5%) and palygorskite (5–8%) (Figure 2). Occurrence of palygorskite (Figure 1) suggests a wide distribution of this clay mineral in the samples. Smectite content (range 42.97–19.94%), generally, shows an offshore-increasing trend (content > 35%), whereas kaolinite content is high (generally 35–40%; at some locations > 40%) along the slope, and off the Karmarna River (Figure 1). Though, on the upper slope illite (15–25%) and chlorite (10–13%) are generally low, along the entire lower slope their contents were found to be higher (illite 25–35% and chlorite > 15%; Figure 1). Gibbsite (~5%) is distributed over the slope region. The results also indicate an absence of < 45 µm size detritus on most of the shelf helping to define a 'no clay zone' (Figure 1).

The climate in the adjacent landmass of the study area is humid tropical – rainfall ranges between 280 and > 400 cm in the inland areas of Western Ghats^{14,15}. The clays reported¹⁶⁻¹⁷ from the soil of the hinterland are

kaolinite and smectite in major quantities with traces of gibbsite. Four small rivers, rising in the Western Ghats (Figure 1) dump their load in this area. The sediments of mud banks and estuarine region (located north of the study area) are reported to have kaolinite and smectite with illite in minor amount¹⁸.

There is a general offshore-increasing trend in the abundance of smectite and kaolinite clays (Figure 1). Owing to the high content of smectite at the southwestern continental margin, Kolla *et al.*⁵ suggested its possible derivation from the Bay of Bengal Waters (BBW). But, illite (80%) and chlorite are the most important constituents of the BBW^{4,19-22}. Smectite too is reported from the adjacent landmass and estuarine sediments^{16,17}, and its content shows more pronounced variations across the slope (< 20% on the upper slope to > 30% in the lower slope) without any reduction in its abundance (~30%) along the slope, particularly in the northerly direction corresponding to the movement of BBW (Figure 1). It is, therefore, suggested that smectite and kaolinite with similar sources and distribution trends, are mainly contributed by the local river discharge and dispersed across-shelf. The presence of gibbsite, forming under humid climate from the weathering in the hinterland³, further corroborates across-shelf transport of the sediment derived from peninsular India. Deposition of illite and chlorite along the southwestern continental margin of India by the low salinity BBW, entering this region

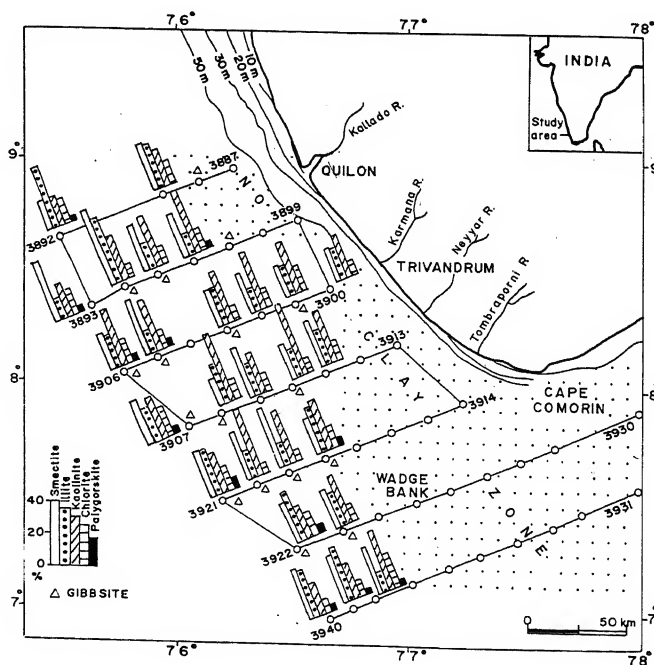


Figure 1. Sampling site, sea bottom physiography and distribution of clays in the sea bed. Note 'No clay zone' in the shelf, and enrichment of kaolinite and smectite on the slope. Distribution of palygorskite in the study area is also shown.

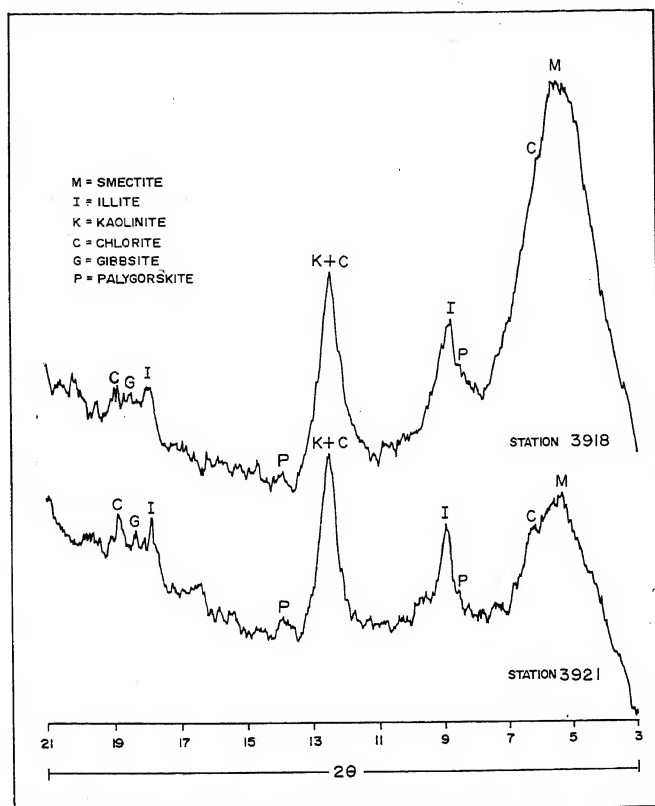


Figure 2. X-ray diffractograms of the sediments from the study area. See Figure 1 for the location of the samples.

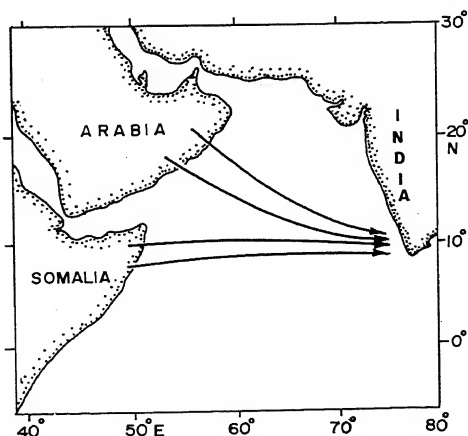


Figure 3. Path of the wind from Arabian peninsula and Somalia.

during November–January^{23–24}, has already been documented¹⁰.

Palygorskite has not been reported from either soil or estuarine sediments though it has been observed over a sizeable area along the slope (Figure 1). Formation

of palygorskite requires saline, semi-arid and hot climate with changes in groundwater level¹¹. Since climate of the hinterland is humid-tropical, formation of palygorskite in this area and its supply from this source appears to be very unlikely. The presence of palygorskite along the slope, therefore calls for a non-local source.

Suitable saline, semi-arid, and hot climate with changing groundwater levels do prevail in the Sebkha and Wadi regions of Arabia¹¹. Palygorskite is also reported from the Mesozoic rocks of the Arabian peninsula and part of Somalia¹¹. In the northwestern and the central Arabian Sea, adjacent to the Arabia and Somalia, content of palygorskite is generally ~10%, which decreases southeasterly and has been attributed to southeasterly dispersal by winds^{5,6,11}.

From Red Sea to Persian Gulf, the Arabian northwesterly winds prevail throughout the year. Aerosol content of these winds is high^{25,26} (3000 µg/m³) and satellite imageries indicate that this dust spreads over the southwest monsoon trajectories in the mid troposphere^{11,27} and its extension has also been observed as far away as south Indian coast²⁸. Palygorskite, besides illite and smectite, is present as one of the significant constituents in the aerosol dust samples^{29,30}. These winds and trajectories of southwest monsoon winds are reported to be the carrier of palygorskite to the northwestern Arabian Sea¹¹. Therefore, in the absence of any local source, and from the foregoing evidences and the satellite data²⁸, it is deduced that the main source of palygorskite lies in Arabia and Somalia. Further, southeastward transport of aerosol dust by Arabian northwesterly winds (as shown in Figure 3) contributes palygorskite to the southwestern continental margins of India.

In the shelf region, a 'no clay zone' has been observed (Figure 1). The prevalent environmental conditions, i.e. high magnitude currents, a large riverine discharge due to heavy rainfall in the catchment areas, and steep river gradient are reported to have inhibited the deposition of the fine silty-clayey particles on the shelf¹⁰. The absence of palygorskite in the shelf, therefore, does not necessarily indicate a weak aerosol source but reflects the role of hydrography which precludes the deposition of fines in the shelf.

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Microstructural evidence for the formation of crenulation cleavage in rocks

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The mica domains of crenulated schists belonging to the Lunavada group of Precambrian rocks in Gujarat show microstructures similar to Schistosité–Cissilament fabric and extensional crenulation cleavage which are features usually developed in rocks from ductile shear zones. While presenting a model illustrating the sequential development of crenulation cleavages and the observed microstructures within cleavage zones, it is suggested that during successive stages of formation of crenulation cleavage, solution transfer is dominant during the earlier stages whereas shearing is important during the later stages.

STRUCTURES such as Schistosité–Cissilament (S–C) fabric and extensional crenulation cleavage (ECC) are commonly known to develop in rocks in ‘ductile shear zones’ (DSZs)^{1–6}. Such structures have also been produced in the laboratory^{7,8}. They form because of a component of extension along a plane of anisotropy². Here we give a description of the microstructures observed in the crenulated schists belonging to the Lunavada Group of Precambrian rocks in Gujarat. These show microstructures along cleavage zones which resemble the structures like S–C fabric and ECC found in DSZs. A model is given to explain their origin during the genesis of crenulation cleavage and the processes operating during different stages of crenulation cleavage development are discussed.

The Lunavada Group of Precambrian rocks occur in the Panchmahal district of Gujarat and parts of southern Rajasthan, India between 22°45′–23°45′ N and 73°15′–74°30′ E (Figure 1). It comprises alternating quartzites and mica schists and belongs to the Aravalli Supergroup^{9–12}. Two episodes of deformation have been reported and the regional metamorphism is dominantly up to the greenschist facies^{10–12}. The rocks are intruded by the Godhra granite. This has resulted in the superimposition of a thermal event over the regional metamorphic event due to heat supplied by the Godhra granite¹³. The present study deals with the microstructures observed in the mica schists.

Mica schists from Vankdi–Vena area lying 18 km east of Lunavada (Figure 1) possess large porphyroblasts of garnet and biotite and show a well developed differentiated crenulation cleavage (S₂). The differentiated crenulation cleavage is formed by microfolding of a pre-existing foliation S₁ and is defined by alternating quartz (Q) and mica (M) domains. Figures 2a, b, e and f show a well-developed differentiated crenulation

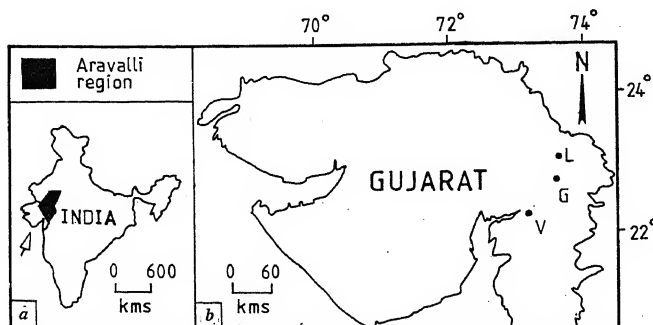


Figure 1. Location map of the study area. Arrow in (a) points to the area shown in (b). L, Lunavada; G, Godhra and V, Vadodra.

cleavage along the limbs of the microfolds. A careful observation of cleavage zones (M domains) has revealed microstructures that resemble those found on the mesoscopic scale in DSZs¹⁻⁶. These are as follows:

- a) In several M domains the S_1 surfaces, defined by mica aggregates, progressively curve sigmoidally and become parallel to the domain boundary (S_2 surface) (Figure 2 a, b, e, f).
- b) In some M domains a cleavage S'_2 develops (Figure 2 c, d, g, h). The important characteristics of S'_2 are: (i) S'_2 forms at a low angle ($<45^\circ$) to the foliation S_2 (domain boundary); (ii) in the domains where it is well developed it imparts a 'button schist' appearance to the M domains; (iii) there is no evidence of metamorphic differentiation during the formation of S'_2 . This suggests that S'_2 defines the real plane of displacement rather than volume loss.

There is a striking resemblance of the microstructures illustrated in the cleavage zones of Figure 2 with those described from mesoscopic scale DSZs. Figure 3 (drawn after Figure 7a of Ghosh⁵) shows the development of S-C fabric in mylonites. Figures 2 a, b, e, f show the progressive sigmoidal curving of S_1 into S_2 which is similar to the S-C fabric found in mylonites illustrated in Figure 3. Similarly, the microstructural features related to S'_2 surface (Figure 2 c, d, g, h) show a resemblance to ECC that have been described in DSZs^{1,2,6}. Significantly, although the mica schists studied here do not belong to a mesoscopic scale mylonite zone or a DSZ, the microstructures observed in the cleavage zones (M domains) of the differentiated crenulation cleavage bear a strong resemblance to the structures found in DSZs¹⁻⁶. This has the important connotation that microstructures resembling S-C fabric and ECC can develop within the M domains of a differentiated crenulation cleavage in mica schists lying outside a mesoscopic mylonite zone. Therefore, it is stated that crenulation cleavage zones can behave as microscale shear zones. During shearing along the cleavage zones, the S_1 , S_2 , and S'_2 surfaces are perhaps similar to S, C and ECC surfaces respectively

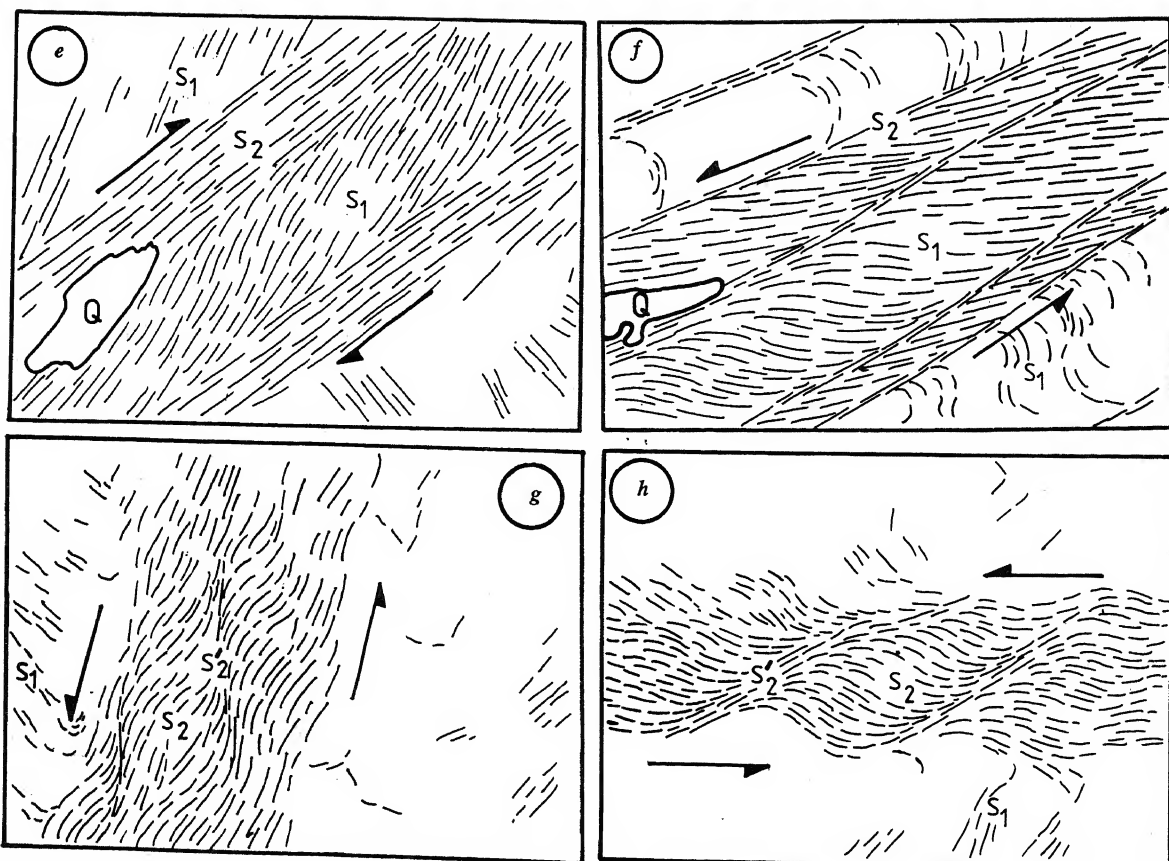
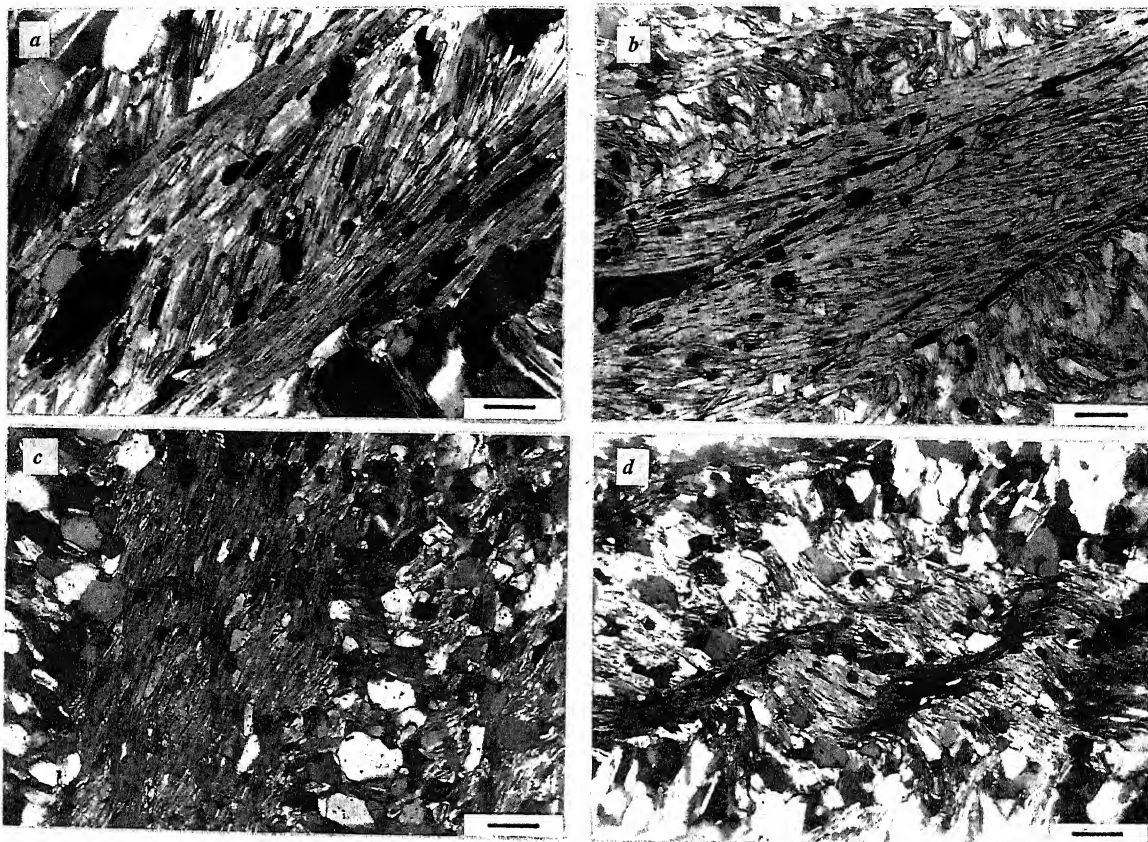
— the latter terms have so far been restricted to describe structures in mylonites.

Based on the microstructures observed in Figure 2, the following sequence of events is invoked for the genesis of crenulation cleavage.

Microfolding of the pre-existing foliation S_1 resulted in the initiation of the differentiated crenulation cleavage (S_2). During the initial stages of microfolding (crenulation), solution transfer resulted in the migration of quartz from the limbs of the microfolds to the hinges, whereas the phyllosilicates got concentrated in the limb regions (see Figure 2 a, b, e, f). This led to the formation of domainal fabric in the rock which is characterized by alternating Q and M domains¹⁴⁻²¹. Volume was lost during this process, at least on the scale of a few microfolds, though this is not essential on a larger scale²². After the rock achieved its domainal fabric, the M domains define a zone of strong anisotropy characterized by S_1 mica aggregates. Shearing occurred along the M domains and this resulted in the progressive sigmoidal curving of the micas defining the S_1 foliation within the M domains into parallelism with the S_2 surface, i.e. the domain boundary (Figure 2 a, b, e, f). The microstructure thus formed resembles the S-C fabric of DSZs. Continued shearing along these domains resulted in the formation of S'_2 surface at a low angle ($<45^\circ$) to the S_2 surface within the M domains of the differentiated crenulation cleavage (Figure 2 c, d, g, h). The S_2 - S'_2 relationship is similar to ECC of DSZs. Volume was conserved during the development of S'_2 because metamorphic differentiation on account of solution transfer had already occurred to a large extent during the earlier stages of the formation of crenulation cleavage (S_2).

Figure 4 (modified after Bell and Rubenach²³) shows a model for the sequential development of crenulation cleavages and depicts the microstructures developed at different stages of their genesis. The rock achieves its domainal fabric by stage 3. Solution transfer is the dominant process up to stage 3. Shearing along the M domains becomes significant from stage 4a and results in a structure similar to S-C fabric found in mylonites. Continued shearing along the M domains results in the initiation of an embryonic S'_2 surface in stage 4b and finally a well developed S'_2 surface develops in stage 4c, which is similar to ECC found in mylonites. With further deformation the S'_2 surfaces in M domains totally rotate into parallelism with the domain boundary and the relic crenulations in Q domains are destroyed. This is stage 5 of crenulation cleavage genesis. Finally, in stage 6 the S_2 foliation gets homogenized.

The present study has highlighted the microstructures observed in cleavage zones (M domains) of differentiated crenulation cleavages in mica schists. These cleavage zones develop along limbs of the microfolds and are



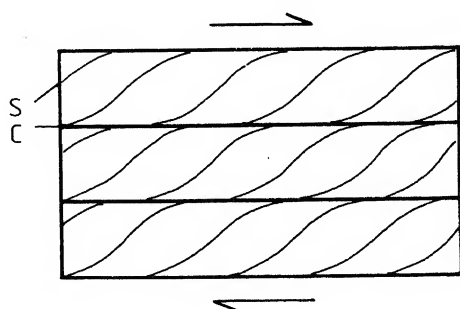


Figure 3. Sketch showing S-C fabric that commonly develops in rocks from ductile shear zones (after Figure 7a of Ghosh⁵).

observed to preserve microstructures resembling S-C fabric and ECC, which normally are found in the mesoscopic scale in rocks from mylonite zones or DSZs. The recognition of these microstructures leads to the revival of the question whether crenulation cleavages are pressure solution surfaces or microscale shear zones. Gray²¹ considered all crenulation cleavages as pressure solution surfaces and stated that no appreciable movement occurs 'at any stage' in their development. Interestingly, the present study has revealed that a crenulation cleavage can behave as both a pressure solution surface and a microscale shear zone at different stages of its deve-

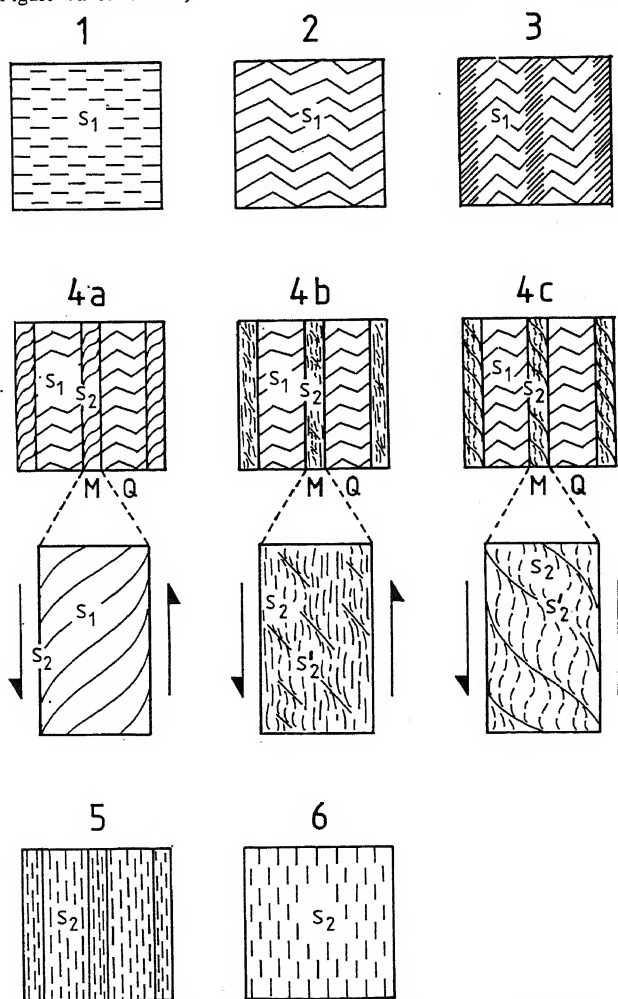


Figure 4. Model showing the sequential development of microstructures in M domains during crenulation cleavage development. Solution transfer and resultant metamorphic differentiation dominate up to stage 3. From stage 4a, the domain boundary starts behaving as a shear surface and results in the development of a fabric resembling S-C fabric which occurs in mylonites. With increasing strain and shearing along M domains, an embryonic S'_2 surface initially develops at a low angle to S_2 in stage 4b and subsequently a well developed S'_2 is formed in stage 4c. The S'_2 resembles the ECC found in mylonites. In stage 5 the relic crenulations in Q domains are destroyed and the S'_2 surfaces in M domains rotate into parallelism with domain boundary. In stage 6 the S_2 foliation gets homogenized. Compare stages 4a, 4b and 4c with Figures 2a, b, c and d respectively. The model is modified after Bell and Rubenach²³.

Figure 2a-h. a, b, Photomicrographs showing microstructure in M domains of a differentiated crenulation cleavage in mica schist. The M domains have developed along the limbs of the microfolds. S_1 mica aggregates in M domains sigmoidally curve into parallelism with the S_2 surface. c, Photomicrograph showing a well developed S'_2 surface within the M domains. Note that the angle between S'_2 and S_2 surfaces in (c) and (d) is less than 45° . (e), (f), (g) and (h) are line drawings highlighting the M domains of (a), (b), (c) and (d) respectively. Arrows show sense of shear. Scale bar is 0.05 mm in (a) and 0.1 mm in (b), (c) and (d). Q is quartz grain.

lopment. Pressure solution (solution transfer) is undoubtedly a dominant mechanism during the earlier stages of crenulation (up to stage 3 in Figure 4) and due to this phenomenon the rock attains its domainal fabric characterized by alternate Q and M domains. However, the presence of microstructures resembling S-C fabric and ECC which commonly occur in mylonites, within the cleavage zones (Figure 2) suggests that crenulation cleavages behave as microscale shear zones during the later stages of crenulation, because metamorphic differentiation has already occurred during earlier stages of the formation of crenulation cleavage. This is in accordance with the suggestion of Williams and Schoneveld²⁴ that the S_2 surface becomes the active surface of shear after all the mobile material has been removed from it.

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Relict coral reef and evidence of Pre-Holocene sea level stand off Mahabalipuram, Bay of Bengal

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A relict coral reef on the outer continental shelf off Mahabalipuram at -115 m depth yielded a radiocarbon (^{14}C) age of 14510 ± 190 yrs BP. A terrace at -130 m depth probably indicates the lowest sea level position during the late Pleistocene. It is inferred that the sea level rose at the rate of 5.71 m/kyr during the early stage of post-glacial transgression between 18,000 and 14,500 yrs BP.

STUDIES document that global sea level has been rising with pauses since the late Pleistocene sea level minimum. Imprints of high and low sea level strands during the late Pleistocene have been well recorded along the west coast of India¹⁻⁴. Although evidences are available about sea level high on the east coast of India⁵⁻⁷, only fragmentary informations about low sea level are available⁸⁻¹³. In this paper, an evidence of Pre-Holocene low strandline from a relict coral reef on the outer continental shelf off Mahabalipuram in the east coast of India has been presented.

Echo sounding (3.5 kHz) data was collected along shore parallel and perpendicular transects on the continental shelf at 10 km interval during R. V. *Samudra Manthan* cruise No. 94. Sediment samples of the mid and outer continental shelf were collected using van Veen grab on 10 km grid. The sediment texture and type were classified. A coral chunk (25 × 20 cm) along with coral debris was collected at -115 m depth (12°33.523'N; 80°37.074'E) off Mahabalipuram (Figure 1). The mineralogy of the coral was determined by X-ray diffraction using CuK_α radiation. The coral was cleaned thoroughly and surface encrustations such as worm tubes and green lichens were removed prior to dating. Radiocarbon age (^{14}C) of the coral was determined by the Birbal Sahni Institute of Palaeobotany, Lucknow.

The continental shelf off Mahabalipuram is about 40 km wide and the shelf break occurs around -135 m depth. Echo sounding profiles (3.5 kHz) reveal the presence of terrace at -100, -110 and -130 m depths, probably related to low strandline positions. Dome-shaped reefal structures occur at about -100 and -115 depths in the outer shelf zone (Figure 2). These features extend parallel to the coast to a limited distance (Figure 1).

The shelf is covered by carbonate-dominated sediments and they are clayey sands with abundant skeletal fragments and ooids and occurs as shore parallel linear patches. Sandy silt and silty clay are also present.

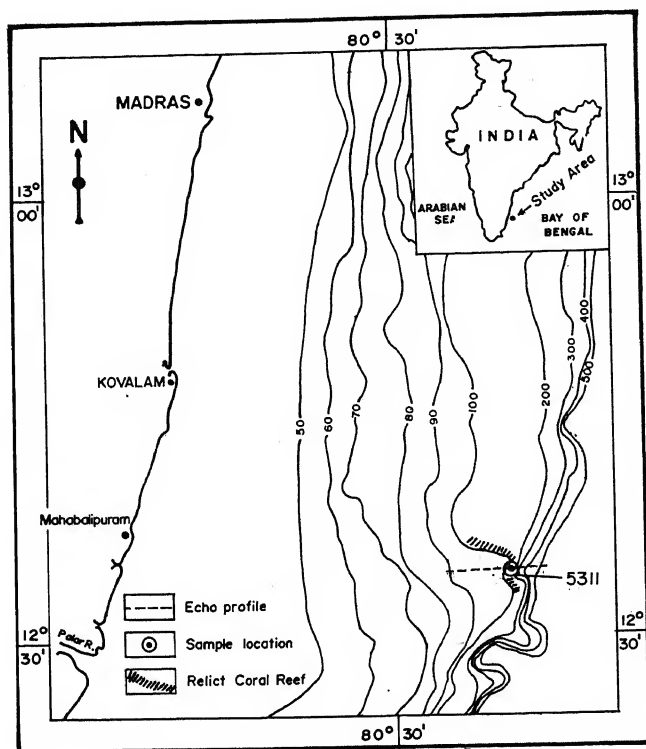


Figure 1. Map showing bathymetry and sample location in the study area.

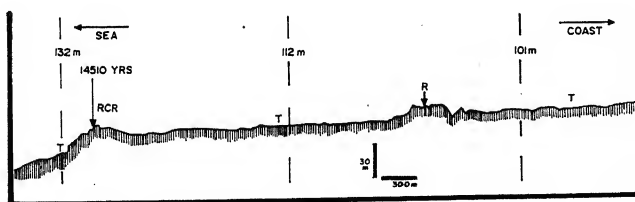


Figure 2. Echo profile (3.5 kHz) off Mahabalipuram showing relict coral reef (RCR), algal reef (R) and terrace (T).

Calcareous concretions are more common in the outer continental shelf sediments.

The coral chunk recovered from the reef at -115 m water depth is sub-massive, hemispherical and corallites are polygonal to circular (Figure 3). The coral belongs to the genus *Goniastrea* sp. Majority of the coral debris are of branching type belonging to the genus *Acropora* sp. The radiocarbon (^{14}C) dating of the coral (*Goniastrea* sp.) yielded an age of $14,510 \pm 190$ yrs (calibrated) BP.

The X-ray diffraction studies on the coral indicate that it contains only aragonite (100%), thus confirming no recrystallization of aragonite.

Reef-building colonial corals thrive in calm shallow waters¹⁴ with temperature of about 22°C . The presence of patchy coral reef at -115 m depth in the study area

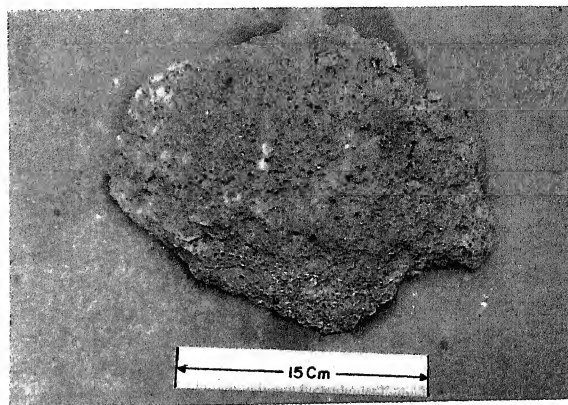


Figure 3. Coral *Goniastrea* sp.

indicates a sea level stand probably at about -110 m depth which facilitated the growth of patchy coral reef at -115 m depth. Further, a lowest terrace feature with ooid concentration was recorded around -130 m depth. This suggests that the lowest sea level during the Last Glacial Maximum (LGM) might have been closer to -130 m in this region. This observation corroborates well with the glacio-eustatic sea level low recorded elsewhere¹⁵⁻¹⁷ at depths between -115 and -130 m.

On the eastern continental shelf of India, terraces at -55 , -60 , -70 , -85 and -100 m depths, probably formed during the Late Pleistocene–Holocene transgression, have been recorded^{9,10-13,18}. Algal barriers at -85 m and -100 m depths off Visakhapatnam have been dated $10,790 \pm 170$ yrs BP and $12,530 \pm 170$ yrs BP respectively¹³. Similarly, the molluscan shells collected from an offshore bar at -17 m depth in Nizampatnam Bay have given an age of 8200 ± 120 yrs BP¹⁰.

It is surmised that the lowest sea level during the LGM was at about -130 m depth in the study area. The coral reef at -115 depth yielded an age of $14,510 \pm 190$ yrs BP. Presuming that the lowest terrace at about -130 m level formed at $18,000$ yrs BP, and there were no pauses in sea level between $18,000$ and $14,500$ yrs BP, it is inferred that the sea level in the study area rose at the rate of 5.71 m/kyr until $14,500$ yrs BP.

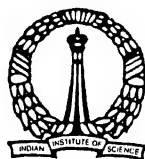
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Indian Institute of Science Bangalore 560 012

Post-Doctoral Research Associateships in Life Sciences and Biotechnology (A DBT-sponsored programme)

Applications are invited from candidates with Ph D in Biological, Chemical or Physical Sciences, or MD, for the award of temporary Research Associateship (maximum duration - 2 years) to carry out research in frontier areas of Biology in the Division of Biological Sciences at IISc under the DBT-sponsored postdoctoral programme. Candidates *who have submitted their theses* (awaiting award of degree) are also eligible to apply, but they may be appointed as Research Associates (Provisional) till they obtain the degree. Minimum consolidated emoluments for Research Associates will be around Rs 4000 p.m. This is an ongoing programme and the PDFs award will be operative from 1 April or 1 October of every year.

Candidates should apply on plain paper giving: (1) Biodata; (2) Summary of Ph D work, including list of publications (reprints if any); (3) Two reference letters from supervisor and persons familiar with their scientific work; and (4) An undertaking that they will continue with the training programme for its duration.

After initial screening, candidates will be called for an interview at IISc., Bangalore in the second week of September 1996/March 1997. The PDF programme will start from 1 October 1996/1 April 1997. Send applications to Prof. K. P. Gopinathan, Chairman, Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore 560 012 to reach him before 20 August 1996/15 January 1997.

Prof. K. P. Gopinathan
Convener
DBT-PDF Programme at IISc

Resonance — Journal of Science Education. N. Mukunda, Chief Editor. Indian Academy of Sciences, P. B. No. 8005, Bangalore 560 080. 1996.

The advances in science have become intractable and mind boggling in recent times. In India, it is very difficult for a teacher to keep abreast of scientific advances due to a lack of an appropriate journal or magazine. In this context, it is heartening to have *Resonance— Journal of Science Education*, which promises to focus among other things on topics that are difficult to grasp and teach at the undergraduate level. It covers a wide spectrum of sciences. It has an attractive layout with a large marginal space imaginatively used to highlight the important messages. In addition to series, general and feature articles, one finds research news and book reviews dealing with the frontier areas of science. The items in the departments 'Classroom', 'Think it over', and 'Information and announcements' are particularly relevant to the teachers. The character of this journal can be appreciated by a selective reading. A few of these are presented here.

The series on cosmology and geometry are very useful and fascinating with the readers eagerly waiting for the next issue. In both these articles, the authors develop the subject methodically and chronologically emphasizing the evolution of new ideas and concepts. The general article on Fermat's last theorem and the article on prime numbers are very interesting. These are informative both for a beginner and for one looking for recent trends in mathematics.

At present, chemistry is taught to convey only a vast amount of descriptive data. It is here that the series on 'Learning organic chemistry through natural products' and 'Fascinating organic transformations' are very instructive. The feature 'Molecule of the month' describes the chemistry of a novel molecule and surely an eye opener to the state of the art in this area. For example, the discussion on cyclobutadiene in a carcerand, i.e. spheroidal molecular prison is very readable and presented in a simple way. This is an important contribution of the Nobel laureate, Donald J. Cram and co-workers. The series 'Life: com-

plexity and diversity' presents in simple language the exciting facets of biodiversity and will kindle the interest of all the students and teachers of science. The general article 'From matter to life: chemistry?' by the Nobel laureate Jean-Marie Lehn is an inspiring account of his journey into chemistry and it is very appealing to the younger generation. In general, the emphasis in chemistry has so far been mostly on organic chemistry. One hopes that in tune with the current trends of inter-connections in all branches of chemistry, the future issues will dwell upon inorganic, bio-organic and organometallic systems as well. The write-up on the 'Honey bee dance language' is very engrossing and has all the make up of a mystery thriller. Under the feature articles, 'Nature watch' dwells upon the exotic and the unusual aspects of life forms in nature. The themes are very impressive and they are illustrated with attractive photographs as for instance, in the article on bats and their diversity. This section undoubtedly is the most popular part of *Resonance*.

One of the important departments of *Resonance* is the 'Classroom'. Here the teacher gets an opportunity to raise questions encountered in the classroom and having bearings on the syllabus material. For instance, in the classroom, Doppler effect is discussed only in acoustics. Its counterpart in light is not emphasized. In this context, the question pertaining to Doppler effect is relevant for a broader presentation of this subject.

Also, the question on Bose condensation of an ideal Bose gas is intellectually stimulating. The teachers will be eagerly awaiting to see the answers to such questions. This section also provides an opportunity to share with the other members of the teaching community, personal experiences and viewpoints related to the teaching and learning of science. In 'Think it over' one can pose puzzles and paradoxes related to the subjects taught. The problem of the weight of a bird in a cage under different situations is very thought-provoking. It is a good example for highlighting the salient points of Newtonian mechanics, like, Newton's third law, free fall under gravity, hydrodynamic lift and frictional forces.

The section on 'Research news' highlights some significant recent developments in science.

Normally it is difficult to recommend good science books to the library in view of the lack of relevant information on their suitability to teachers and students. In this context review of recent books and reprints of classic books are very helpful.

It is worth mentioning that not all the articles are at the same level of lucidity. Some of them are really difficult to understand. Generally, the presentation of materials under research news is difficult to comprehend. Further, though *Resonance* is termed as a journal of science education, the educational component of the material is generally not found in many of the articles. Only in the presence of such links, one can understand and appreciate fully the subject matter of the articles. By way of a discordant note, one notices some minor irritants in the nature of typos and inadvertent errors. One hopes that the future issues will be free from such blemishes.

We would like to take this opportunity to make a general comment on science education. Invariably, in our schools and colleges excessive emphasis is laid on learning by rote instead of understanding the concepts. Therefore a student who is successful in our examination system does not necessarily possess a grasp of the subject. This calls for a reassessment of our teaching and evaluating procedures. *Resonance* hopefully will act as a catalyst for reforms in science education.

In our view, another journal of this quality and content, does not exist in India. It certainly fills a void in the educational system of our country. Students and teachers stand to gain considerably in the understanding and appreciation of science. The Indian Academy of Sciences has done yeoman service to the community of teachers and students by bringing this out at an easily affordable price. *Resonance* is a valuable reading material to every teacher and is a must in all the college libraries.

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BOOK REVIEWS

Annual Review of Earth and Planetary Sciences 1994. Vol. 22. George W. Wetherill, ed. Price: USA \$ 62, elsewhere \$ 67. 691pp; **Annual Review of Earth and Planetary Sciences 1995.** Vol. 23. George W. Wetherill, ed. Annual Reviews Inc., 4139 El Camino Way, P. O. Box 10139, Palo Alto, California, USA. Price: USA \$ 62, elsewhere \$ 67. 513pp.

The recent volumes of *Annual Review of Earth and Planetary Sciences* carry in-depth reviews written by experts in their respective areas of specialization, and present a wide spectrum of research topics, ranging from evolutionary history of whales and dolphins to tectonic and magmatic evolution of Venus. Some of them are currently active topics, while others deal with emerging fields. As Frank Press rightly points out in his introductory article (vol. 23), the golden age which had witnessed extraordinary creativity and discovery may have been over, but the science and its methods will be appropriately remoulded to suit the new age. The articles under review in fact testify to the reshaping of science to suit the demands of a new era.

The volumes reviewed here contain eighteen and fifteen articles respectively on varied topics, with some overlap. The articles, viz. 'The fate of descending slabs' by Thorne Lay (vol. 22) and 'The mechanics of deep earthquakes' by Harry W. Green II and Heidi Houston (vol. 22) deal with similar problems. But the reader is benefitted because of their differences in perspective. The discovery of deep focus earthquakes should be considered as momentous, as they had made some lasting contributions to the earth sciences. Major questions still remain as to the mechanics of the deep earthquakes in the subduction zones, and also what happens to the descending slabs of oceanic crust. Answers to these questions which have implications for mantle convection, are now sought not only from seismology, but also from high pressure mineral and rock physics.

The articles, 'Quantum geophysics' by M. S. T. Bukowski (vol. 22) and 'Effects of phase transitions on mantle convection' by Ulrich Christensen (vol. 23), also address questions related to inner earth environments. The article on

quantum geophysics focuses on the advances made in the theoretical mineral physics, and presents a case as to why the diverse internal properties like structure compressibility, phase transformations, vibrating and optical spectra and rheology that are interrelated at the atomic level, have to be conceptualized and unified from a theoretical point of view of quantum mechanics, especially when the conditions that exist in the inner Earth may be difficult to simulate by the available laboratory techniques. The second paper reviews the role of phase transitions in influencing mantle convection. A larger question, however, is the relative role of mass flux between upper and lower mantle. This is an important problem in earth sciences from another angle. Although the plate tectonics is the accepted paradigm to account for stress accumulation and crustal deformation, it is increasingly realized that the creeping flow of mantle rocks by buoyancy forces due to thermal expansion has a role in generating stresses in the continental crust. The article by Ulrich Christensen presents the results of numerical modelling to resolve the question of nature of mantle convection, and concludes that a compromise of two end member models of whole-mantle convection and layered convection may be plausible.

Palaeoclimatic changes form the theme of many papers in these volumes. 'Late Eocene-Oligocene extinction' by Donald R. Prothero (vol. 22); 'Palaeoclimatic estimates from Tertiary leaf assemblages' by Jack A. Wolfe (vol. 23); 'The initiation of northern hemisphere glaciation' by M. E. Raymo (vol. 22) and 'Sequence stratigraphy' by Nicholas Christie-Blick and Neal W. Driscoll (vol. 23) are the articles under this theme. The first article focuses on the transition from the Eocene to Oligocene Epochs. Improved dating and correlative techniques which increased the resolution of data suggest a protracted pattern of Eocene-Oligocene extinction, precluding any sudden impact events. The review of Tertiary leaf assemblages covers the application of the 'Climate-Leaf Analysis Multivariate Program' (CLAMP) to assess the Tertiary nonmarine climate and its results from some selected areas. This statisti-

cal approach aids in analysing the Tertiary leaf varieties induced by climatic factors. Results show a 'terminal Eocene temperature deterioration' in the continents as well. The fourth article covers the science of sequence stratigraphy. The sequence stratigraphy is relatively a new discipline systematized in the 1970s when Exxon, the oil giant, released its seismic reflection data from the edges of the continents, much to the excitement of scientists world over. From these data, the scientists could decipher the pattern of the rise and fall of sea level over the past 250 million years. These studies have churned out voluminous output in the interpretation of global sea level falls. However, questions remain as to the relative role of tectonic processes and climatic factors (fluctuations in the size of ice sheets) in forcing the coastal onlaps. It is expected that recent Ocean Drilling Program (ODP) in the coastal waters may come up with complementary data. The role of tectonism is emphasized in the article on northern hemisphere glaciation by M. E. Raymo. He examines the climatic transition from warm mid-Pliocene (~3.2 Ma) to the onset of northern hemisphere glaciation around 2.4 Ma. Resolving climatic patterns of the recent geologic past is important, particularly because an increase of 3°C is predicted for the next century.

Among the review articles in the two volumes, one of my favourites is the article titled 'Arc assembly and continental collision in the Neoproterozoic east African orogen: Implications for the consolidation of Gondwanaland' by Robert J. Stern (vol. 22). The impressive style of narration and the brilliant exposition of ideas make it a memorable article. The Neoproterozoic Era (1000–540 my), which encompassed a protracted orogenic cycle, is a fascinating interval characterized by the evolution of eukaryotes and appearance of metazoa, large events of continental glaciation, increased concentration of oxygen in the atmosphere and the development of banded iron formation. Pan-African Orogenic cycle was not restricted to Africa, but throughout the Gondwanaland. The evidences of the Pan-African tectonism indicate that the plate tectonic systems had been in ex-

istence even in the early phases of the Earth's history. The article chronicles the sequence of tectonic events comprising the East African Orogen (EAO). Of special interest is the mention of granulites of southernmost India and Sri Lanka, which were earlier thought to be Archean and Paleoproterozoic, but have since been proved to be Neoproterozoic (660–550 Ma). The author suggests that the younger ages indicate a second younger collision event between the west and east Gondwanic continents. He further states that collision along EAO led to the crustal overthickening and development of strike-slip shear zones and faults related to extensional basin. The modern analogue is the 'escape tectonism' observed in parts of the Himalayan orogen.

Reading this type of compendiums gives you an experience akin to a space mountain ride in Disney World. From a breathless ride in the realm of Neoproterozoic world, we are thrown into a mundane world of earthquakes and active tectonics, and again to much rarefied fields of tectonic evolution of Venus, radar investigations of Mars, Mercury and Titan and physics of zodiacal dust and the mind-shattering enigma of origin of life. I like to focus on the chapter on earthquakes and active tectonics first, being the topics that are closer to my heart. H. Kanamori gives a succinct review of the physical processes of earthquake generation in his article on 'Mechanics of earthquake generation' (vol. 22). There have been significant advances made in the study of slip distribution on a fault. Seismic imaging of major earthquake zones shows that slip models can be interpreted in terms of 'barriers' and 'asperities', ideas to which Kanamori himself has made significant contributions. He emphasizes the mechanical models of faulting involving the factors such as macroscopic and microscopic static stress and dynamic stress fields. The article on 'Active tectonics of the Aegean region' by James Jackson (vol. 22) deals not only with earthquakes, but also tectonics. What is most interesting here is the abundance of observational data, precluding any element of speculation. The whole dynamics of the deformation is built upon from the high quality seismicity data

and measurements of velocity field in the fault zones using space-based geodetic techniques.

Christopher F. Chyba and Gene D. McDonald review some current issues related to origin of life and exobiology research (vol. 23). Consensus is that *sine qua non* of life is liquid water. Thus, search for life is essentially a search for liquid water. This view is criticised as being born out of 'parochialism' by a group led by irrepressible Carl Sagan. An alternate view insists that extraterrestrial life may look quite different from what we are accustomed to. The article also brings out some salient issues concerning the origin of life on Earth. The questions on the sources of energy available to force the prebiotic organic synthesis are also addressed. A 'chicken or egg' problem haunts the researchers in this field. Which formed first: RNA or proteins? The majority favours RNA. On the whole, the authors have done a thorough job and leave out nothing on one of the most engaging areas of interdisciplinary research today.

Some reviews deal with emerging fields. What appeared most exciting to me in this category is an article titled 'Geomorphology and *in-situ* cosmogenic isotopes' by T. E. Cerling and H. Craig (vol. 22). This article presents an avantgarde method to date the geomorphologic surfaces using the production rates of different cosmogenic isotopes. This method of dating the erosional surfaces will help to resolve some of the long-standing problems in geomorphology, archaeology and active tectonics. The work of an Indian scientist, D. Lal finds repeated reference in this paper. Fortunately, a solid infrastructure and a strong tradition exist in our country that is helpful to strengthen and diversify this line of research to solve some specific problems.

The annual reviews also contain articles in the domain of planetary sciences, in particular about radar imaging of Mars and Venus. Other chapters in the volumes cover a potpourri of topics. As no book review is considered in order without a few quibbles, I would like to add that a topical arrangement of articles would have made these volumes more reader-friendly. On balance, I must say that these articles open up wide

vistas of future research, and remind us most emphatically that the earth sciences have really come of age into a world of high quality data, high resolution and increased quantification of natural processes. And, they are recommended to both professionals and students for these reasons.

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Biology of the Fungi by Dr J. G. Vaidya. Satyajeet Prakashan, I Floor, Sulochan Apartments, Pune 411 038. 1995. I Edition. 670 pp. Price not known.

Fungi have been getting increased recognition as important components of the microbial world and presently global attention is focused on aspects of biology, biochemistry and biotechnology of fungi for enhancing our knowledge base essential for achieving progress. In this context, a book dealing with Biology of Fungi to provide authentic information on the subject is most welcome.

The book on the *Biology of the Fungi* attempts to give an overview of the salient aspects of fungi under two major sections, viz. Fundamentals (Part I) and Fungi in Action (Part II). Topics dealt with under the Fundamentals section include biological characters, vegetative growth and reproduction, ultrastructure and nutritional aspects. In the second part a wide range of topics including fungal pathogenesis, nutrition and metabolism and role of fungi in biodeterioration and biodegradation are covered. Two chapters are also devoted to fungal metabolites and role of fungi in biotechnology.

The author has stated that he has chosen a 'multidisciplinary approach for making fungi comprehensible to the layman' while the references are included for the sake of 'students and research workers'. Each section comprises of six chapters and the contents of each chapter are presented under many subtitles.

While appreciating that the author has expended considerable effort in compiling information pertaining to fungi and presented them in a manner

BOOK REVIEWS

that he has felt optimal for meeting the requirements of his target readership, it appears that for the uninitiated reader, the text is somewhat disorganized to get a comprehensive picture of biology and biotechnology potential of the fungi. For example, the Section I in Chapter 2 deals with heterotrophic carbon nutrition before the aspects of vegetative growth, reproduction and ultrastructure are presented. Nutrition and metabolism including metabolic pathways of carbon utilization are described in Section II. Fungal ecology and distribution (Chapter 8, Section II) should have come in the first section since it cannot be adequately justified to find a place under Fungi in Action. In providing the subtitles and making the descriptions brief or replacing the text with several tabular statements, the author seems to have made it somewhat difficult for gaining a full appreciation of the subject, particularly to the inexperienced student of mycology and fungal biotechnology. There is a paucity of vital information in the text on topics such as fungal biodiversity, selective techniques for pure culture isolation, conservation and identification, genetic

variation in *in vitro* cultures, sporulation, dormancy and mechanism of survival in the natural environment. In the area of genetic engineering involving fungi (page 512), for example, no reference is made to the recent spectacular developments of using filamentous fungi as hosts for heterologous expression of eukaryotic and mammalian proteins like chymosin. That fungi are increasingly becoming preferred hosts in molecular biology due to their efficient protein-secreting potential has also not been mentioned.

In the chapter on Biotechnology, it is surprising that gibberellins are treated under non-industrial products and the description of the whole fermentation is mentioned in just a few lines giving only trivial and insignificant details (page 564). Likewise the description of β -carotene fermentation (page 562) by mucoraceous fungi without detailed mention of the involvement of heterothallic strains and formation of zygospores is a serious omission in a treatise dealing with biology of fungi.

Several additional discrepancies can be listed but it is adequate to state that the text while providing information on

several aspects of fungal metabolism, biochemistry and biotechnology lacks the essential emphasis on those critical aspects which will enable the reader to obtain a comprehensive picture of the biology and biotechnology of fungi. Several errors (e.g. 'hemicellulose is half cellulose molecule' (page 22) and reference to Fig. 2.1 (cellulose) under hemicellulose), spelling mistakes and ambiguous construction of sentences/descriptions (e.g. comparison of fungi and ant colony under social organization (page 11)) also deter the value of the book to a considerable extent. References cited to original papers include many which are not readily accessible to the readers. Perhaps references of relevance to some authoritative reviews on special topics in Annual Reviews etc. would have been more helpful and useful to the readers.

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CENTRE FOR ECOLOGICAL SCIENCES INDIAN INSTITUTE OF SCIENCE

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Last date for receipt of applications is 31st August 1996. The cost of return train fare and boarding and lodging in Bangalore will be borne by the Organizer.

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GOVERNMENT OF INDIA
Ministry of Science & Technology
Department of Biotechnology (DBT)

BIOTECHNOLOGY NATIONAL ASSOCIATESHIP SCHEME: 1996-97

Applications are invited from Indian nationals for the award of Biotechnology National Associateship for pursuing advanced research or undergoing specialized training in Indian research institutes/laboratories in the priority areas of Biotechnology, viz. (i) Molecular biology; (ii) rDNA technology; (iii) Immunology and immunodiagnostics; (iv) Animal cell and tissue culture, hybridomas and cell-culture based vaccines; (v) Plant tissue culture; (vi) Animal biotechnology; (vii) Aquaculture and marine biotechnology; (viii) Peptide and nucleic acid chemistry; (ix) Modern techniques in industrial microbiology and bioconversion; (x) Biochemical engineering, downstream processing and bioseparation techniques, computer modelling and process optimization; (xi) Bioinformatics, and (xii) Emerging areas.

The applicants should possess a Ph D/MD degree in areas related to biotechnology. The candidates should hold regular positions in research institutions and should be actively engaged in biotechnological R&D work. Applications from persons working on temporary positions such as research fellows or associates will not be considered. Scientists/technologists belonging to DSIR recognized in-house R&D centres under the aegis of public or private sector units, intending to join public-funded research institutes/national laboratories/universities and vice-versa, i.e. scientists from the latter institutions, joining in-house R&D centres may be given some weightage for the award, provided they propose to work on specific scientific/technological problems relating to the production of Biotechnology products.

Age limit: 40 years; Candidates should have not crossed this age limit as on 1 January 1997.

Details of the award

Duration: Six months to one year. In deserving cases, the duration is extendable by a maximum period of one year. In addition, the Associates who avail the award for a period of nine months or more in India would also be considered for three months training abroad.

Approval for the overseas training (where admissible) will not be automatic and will be decided on the basis of progress made by the Associates in their work within the country under the award, proper justification, and recommendations of his/her Indian supervisor as well as the associates' parent institute. The overseas training can be availed of within two years of completion of the research work/training within the country under the award.

Associates are entitled to (a) monthly associateship of Rs 2500 while in India and US \$ 1200 or its equivalent during the overseas training; (b) disturbance allowance towards housing in India on actual rent basis subject to maximum limit of Rs 500 per month; (c) personal equipment grant of Rs 2000 (lumpsum), provided the Associateship is availed in India for at least six months and, in addition, a personal equipment grant of Rs 2000 for overseas training; (d) Research contingent grant of Rs 10,000 for six months period of the award and then Rs 5000 for every three months, which is disbursed to the host institutes; (e) first class rail fare and air passage cost in economy class for joining host laboratories in India and abroad respectively and back; (f) the Associates availing the award in another institute located at the same station of the host institute will not get the monthly associateship and disturbance allowance, except the personal equipment grant and research contingency grant.

The selected candidates shall be entitled to the payment of salary and other service benefits by the institutions to which they belong. However, no liability on any of this account will be borne by DBT.

Every selected candidate shall be required to execute a service bond to serve in India for at least one year after completing their training under the award, but in case of the overseas training, the bond's period will be two years and the bond is to be executed in supersession of the earlier bond.

Selected candidates should commence work under the award before 31 December 1996.

Candidates should submit their applications typed on plain paper (six copies with single enclosures) in the format given below. No advance copy will be entertained. Candidates should ensure that application through proper channel should reach Shri R. N. Mehrotra, Director, Department of Biotechnology, Block 2, CGO Complex, Lodhi Road, New Delhi 110 003 by 15 September 1996. After completion of selection procedure results of selection will be communicated during December, 1996.

FORMAT

Application for the Biotechnology National Associateship (199-97)

(1) Applicant's name (in full), designation and address (2) Father's/husband's name (3) Date and place of birth and age (4) Academic qualifications (degree onwards with subjects of specialization and distinction(s), if any) (5) Past and present employment record (give details about employer(s), position(s) held and its nature - temporary/permanent, duration, duties and pay in chronological order) (6) Subject of current research work (enclose details of the research work being pursued by the candidate) (7) No. of research publications during the last five years (enclose list and attach reprints of three research papers which you consider the best) (8) Proposed subject of research/training (enclose (i) the precise research/training programme to be undertaken during the award, (ii) its relevance to the priority areas identified by DBT, (iii) to the plans and programmes of the parent institute, and (iv) candidate's future research plan for utilizing the knowledge gained during the award) (9) Proposed place of research/training (has the consent of host-institution been obtained?, if so, attach the consent letter) (10) Period for which the associateship be availed (11) Duration and other details of the past overseas visits, if any (12) To which DBT identified priority area, the proposed research/training programme belongs.

Place

Date

Signature of the Candidate

STATEMENT FROM THE PRESENT EMPLOYER

(As per the terms and conditions of the associateship, candidates selected for the awards should be granted deputation terms such as entitlement to the payment of full salary and continuation of other service benefits during the period of the associateship by their employers. The parent institutions/employers must indicate clearly their commitment to this effect as also about continuity of employment after the associateship period. Applications which are forwarded without such statement may not be considered. Whether the candidate is on regular or permanent staff of the institutions must also be indicated in the statement.

NOTE: (1) The Associateship is tenable only at those institutions which are engaged in major biotechnological research programmes. The DBT has identified the following list of such institutions: BARC, TIFR, HLRC, CRI, IIT, Haffkins Institute, Bombay; AIIMS, JNU, DU, CFB, IARI, IIT, NII, Delhi; NCL, NIV, HAL, Poona University, NFATCC, Pune; IICB, Bose Institute, Jadavpur University, Calcutta; IVRI, Izatnagar; IISc, Bangalore; Anna University, IIT, Madras; IIT, Kharagpur; CCMB, Hyderabad; IMT, Chandigarh; CFTRI, Mysore; CRI, Kasauli; RRL, Jammu; CDRI, NBRI, CIMAP, Lucknow; MKU, Madurai; BHU, Varanasi; MSU, Baroda; Roorkee University. However, other institutions having well-established lab facilities and working actively in areas of biotechnology may also be considered in this regard. Candidates should correspond themselves with their host-institutes for their placement. (2) The Associateship is not tenable at the candidate's parent institutes. (3) Applications not sponsored by employer and forwarded by the institution/organization or received after the last date are likely to be rejected.

CURRENT SCIENCE

A fortnightly journal of research

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R(IA) 308–6/96

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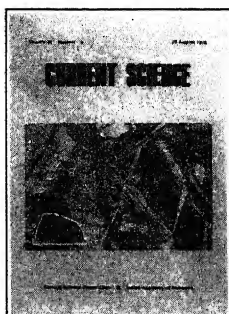
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COVER. The Pipliya meteorite under microscope showing brecciation and intergranular texture. Inset: A fragment (Pipliya-2) of the fall. Photos: M. S. Sisodia. See page 253.

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In this issue

Plants as communicators

It was many decades ago that J. C. Bose attempted to frustratingly educate the reluctant biologists and in particular the conservative botanists about the ways in which plants could feel, respond and even communicate. What he earned to begin with, was mostly ridicule. Gradually, however, the reluctance gave way to curiosity, and the ridicule was replaced by sympathy if not apology. The conservative view that plants are different from animals did erode and they were viewed just as a continuum in the spectra of living things created and shaped by the Darwinian forces of natural and sexual selection. There are a lot more botanists today who are willing to accept plant ethology as a field with strong conceptual basis and without the stigma of anthropocentric views; it has been realized that these sessile organisms could in fact feel, respond and communicate in their own way. For instance, plants attacked by the defoliating insects are known to emit volatile chemicals which induce other plants in the neighbourhood to synthesize the antifeedants in order to deter the defoliators – a kind of warning process where the potential victims are signalled against the impending dangers of defoliation.

Interestingly, certain defoliating insects themselves seem aware of such strategies of the plants much more than some botanists are willing to accept that plants could indeed do so. It has been shown that certain cucurbits when attacked by the defoliating insects send the signal from the parts that are fed upon (leaves) to other parts of the plants from where the antifeedants are synthesized and translocated to the part of infestation (leaf) such that further damage by the pest is minimized. However some defoliators are known to first girdle the petiole of the leaf before they start feeding such that the antifeedants from other parts of the plant do not reach the target leaf. Consequently, the insect could de-

vour the whole leaf without any defence by the plant.

One interesting question that obviously emerges is how fast and how does the plant feel the defoliation by the insect and how is the signal transferred to other parts of the plant. Sashidhar and his colleagues at the University of Agricultural Sciences, Bangalore and Sinha and his group at Indian Agricultural Research Institute, New Delhi (Sinha is now in the Water Technology Centre, IARI) have been investigating exactly such details of signal transfer within the plants in a different context – they are studying the mechanism of signal transfer under conditions of drought stress to the plants. Sashidhar's group is evaluating the possibility of abscisic acid (ABA) as a signal emerging from roots subjected to moisture stress while Sinha's group is tracking the electric pulses as potential signals between stressed roots and leaves above ground.

Consider a plant experiencing the moisture stress. It is very essential that the plant shifts its physiology from a highly metabolic state towards a state that conserves its water while maintaining the minimal life processes. This shift requires that the stress experienced by the roots below the ground is conveyed to the leaves above the ground. These signals help the leaves close their stomata to minimize the transpiration through which much of the water loss occurs in leaves. Such message transfer has to occur as rapidly as possible such that not only could the plants minimize their metabolic activity following the moisture stress, but also resume their normal physiological activity following water loss. Further the signal transferred should also carry the information regarding the extent of the moisture stress in order that the response of the leaves is in proportion to the severity of the stress. Rekha and others from the Sashidhar group at University of Agricultural Sciences, Bangalore, review (page 284) the merits and constraints associated with the ABA and electric pulses as

signals in such processes. They narrate the difficulties of creating the moisture stress as a single signal-emitting process in such studies. It is not unlikely that the groups might look for other stresses such as defoliation of leaves in species such as cucurbits where probably the induction of signals could be much more sharp. In any case their study is one step further towards viewing plants not different from animals in their skills of communication and behaviour.

K. N. Ganeshaiah

Solvent effects

Most chemical reactions are carried out in solution, with solid state and gas phase reactions being practised by only a small population of chemists. While considerable attention is usually paid to the structures and stabilities of reactants and products, the subtleties of the solvent medium are usually conveniently ignored. At times clever manipulation of solvents can result in dramatic enhancements of reaction rates and product yields.

A notable example is the use of aqueous solutions to carry out Diels-Alder reactions. In water, salt effects can be striking. For example in the synthesis of the natural product cantharidin ultra high pressures (16–18 kbar) were found to be necessary. The use of lithium perchlorate in diethyl ether results in efficient synthesis at atmospheric pressure. Can such solvent effects be rationalized? Anil Kumar (page 289) reviews studies that suggest that 'internal pressure' of a salt-solvent system can serve as a measure of the electrostriction effect. Taken together with activation volumes for reactions, this quantity affords insights into the causes of the observed rate accelerations. The author extends these ideas to an apparently unrelated, but interesting problem – the thermal stabilities of DNA

duplexes. Spectacular changes in melting temperatures of double helical DNA occur in the presence of salts. The treatment of these solvent effects discussed in the article by Anil Kumar departs significantly from conventional wisdom. Rational 'solvent engineering' to control reaction rates, stereoselectivities and thermal stabilities may indeed be possible in the not-too-distant future.

P. Balaram

Visibly spectacular pressure-induced transitions in copper metagermanate

Copper metagermanate (CuGeO_3) is a very soft layer-type material like mica with a light blue colour and crystallizes in the orthorhombic space group Pbmm (C_{2h}^2). The discovery of spin-Peierls transition below 14 K at ambient pressure by Hase, Terasaki and Uchinokura (*Phys. Rev. Lett.*, 1993, **70**, 3651) has generated considerable interest in this system. It is of interest to note that CuGeO_3 is the first inorganic system to exhibit the exotic spin-Peierls transition when cooled to low temperatures.

Recent high pressure investigations by Jayaraman and co-workers at the University of Hawaii (*Phys. Rev. Lett.*, 1995, **75**, 2356; page 306, this issue) have shown that this system exhibits several pressure-induced phase transitions. The novel aspect of the high pressure behaviour is the spectacular colour changes associated with these phase transitions. These authors have carried out extensive high-pressure investigations on this system using Laser Raman Spectroscopy and optical microscopy as diagnostic tools in the conventional diamond anvil pressure apparatus. Three distinct high-pressure phases are observed in the

7–9 GPa (70–90 kbar) region when the normal orthorhombic phase (phase I) of CuGeO_3 is compressed. However, the type of phase obtained depends sensitively on the hydrostaticity of the pressure transmitter employed in the studies. Thus when a gas like helium or a liquid like 4:1 methanol/ethanol mixture is used as a pressure transmitter (which ensures a near hydrostatic pressure environment for the sample), the sample as observed by an optical microscope contracts abruptly by nearly 15% in the b -direction near 7 GPa. This new high pressure phase labelled phase II with a striking contraction in the b -axis direction has a pale green colour and a Raman spectrum different from that observed in phase I. The reverse phase transition occurs near 5.6 GPa on pressure release. It is to be emphasized that helium has been established to be a perfect hydrostatic medium up to 11.8 GPa at room temperature and methanol (which freezes just a little above 7 GPa) provides a similar environment for the sample.

The usage of argon which freezes at a relatively low pressure of 1.2 GPa at room temperature leads to a rather complex behaviour. The normal orthorhombic phase (phase I) on pressurization turns blue near 7.5 GPa (designated as phase III). Even as this transition is progressing, these authors observed yet another phase transition (phase IV) accompanied by a colour change from blue to green. It may be remarked that each of these phases has a characteristic Raman spectrum. The green phase (phase IV) which is stable up to 15 GPa reverts to the blue phase (phase III) near 5 GPa on pressure release and further can be quenched to ambient pressure. The quenched phase transforms to the original orthorhombic phase only on heating to 600°C.

These visibly spectacular pressure-induced phase transitions with a

rich variety of phases in a non-hydrostatic pressure environment pose several interesting possibilities. The authors point out that the structure of copper metagermanate is closely related to the pyroxene family minerals but with an important difference. The backbone structures consisting of corner shared GeO_4 tetrahedra in this system lie on the same side of the chain while they lie on either side in a pyroxene chain. The stability of this chain is rather low because of the strong repulsion of the highly charged Ge^{4+} ions. This consideration has prompted the authors to propose that this delicately balanced chain structure transforms to the stable pyroxene type of structure at high pressures. Although the structures of these high pressure phases have not yet been determined, the blue phase is speculated to have the pyroxene type of chain structure. Since the blue phase can be pressure quenched, structural work on these samples would confirm or otherwise this conjecture.

The observation of the pale green phase (phase II) in truly hydrostatic media is perhaps the most interesting aspect of this work. Raman spectra of this phase seem to confirm that the chain structure is preserved during the phase transition. The abrupt decrease in the b -axis accompanying the transition is possibly related to a rotation of the chain in the structure without breaking any bonds. The transition from phase I to phase II is observed only when the sample is unconstrained (true hydrostatic medium) while the transition proceeds in a complex way when the sample is constrained in the non-hydrostatic pressure medium. The authors speculate that the phase II is a ferroelectric-ferroelastic phase. Further work on this system should throw more light on these fascinating possibilities.

T. G. Ramesh

CURRENT SCIENCE

Volume 71 Number 4

25 August 1996

CORRESPONDENCE

Patterns of creativity in arts and sciences

This refers to the lecture entitled 'Shakespeare, Newton and Beethoven or patterns of creativity' by S. Chandrasekhar (reprinted in *Curr. Sci.*, 1996, **70**, 810). About the contrast in the patterns, Chandrasekhar remarks, '... two facts emerge with startling clarity: the remarkable similarity in the creative patterns of Shakespeare and Beethoven on the one hand, and their stark contrast with that of Newton on the other.' At the age of forty-seven Beethoven said, 'Now I know how to compose.' Chandrasekhar writes 'And this to my mind is the center and the core of the difference: the apparent inability of a scientist to continually grow and mature.' In another article, 'Beauty and the quest for beauty in science' (*Phys. Today*, July, 1979) Chandrasekhar makes his point clearer. An artist '... ascent to higher peaks of accomplishment. But I am not aware of a single instance of a scientist of whom the same can be said. His early successes are often his last successes.'

Chandrasekhar has mentioned only one feature in which a sharp contrast

exists. We point out a few more, in which we find equally striking contrast.

All artistic creations have some form of perfection associated with them. The slightest modifications impair their beauty. Nobody can produce an improved version of Shakespeare's play or Beethoven's symphony. But a scientific theory grows continuously from the contribution of other scientists.

Original works of arts do not lose their beauty with time. People read and enjoy Shakespeare's plays even today. On the other hand original works of science gather dust on the shelves.

The ecstatic joy that a scientist experiences when he discovers a new fact or a new pattern or symmetry in nature, is often compared to the aesthetic feeling generated by a work of art. But there is a striking difference. When the new discovery becomes a part of the scientific knowledge, it loses the capacity to excite mind in the same way. Only the discoverer seems to have the privilege. But a work of art continues to trigger sensitive minds to ecstatic rapture long after it was first created.

Discovery of surprising new facts may excite the mind to ecstasy. But nobody, I believe, would credit an experimental discovery as having aesthetic elements. On the other hand, Einstein's general theory of relativity is almost universally acclaimed as endowed with exceptional aesthetic qualities. This is, however, not true for all theories. About quantum mechanics Gellman remarked, 'Quantum mechanics – that mysterious, confusing discipline which none of us really understands but which we know how to use'. We wish to conclude by putting a small question mark after Chandrasekhar's emphatic statement '... in the arts as in sciences, the quest is after the same elusive quality: beauty.'

S. SENGUPTA

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SCIENTIFIC CORRESPONDENCE

The Pipliya meteor shower: A preliminary study

The meteorite which was seen to fall on 20 June 1996 at 2030 hours by local residents at village Pipliya Kalan (lat.

26°2'5" long. 73°56'30"), Pali, Rajasthan, India has been studied preliminarily and hereby named as the

Pipliya meteorite. The present report is the first technical note on the meteorite. The location of the fall

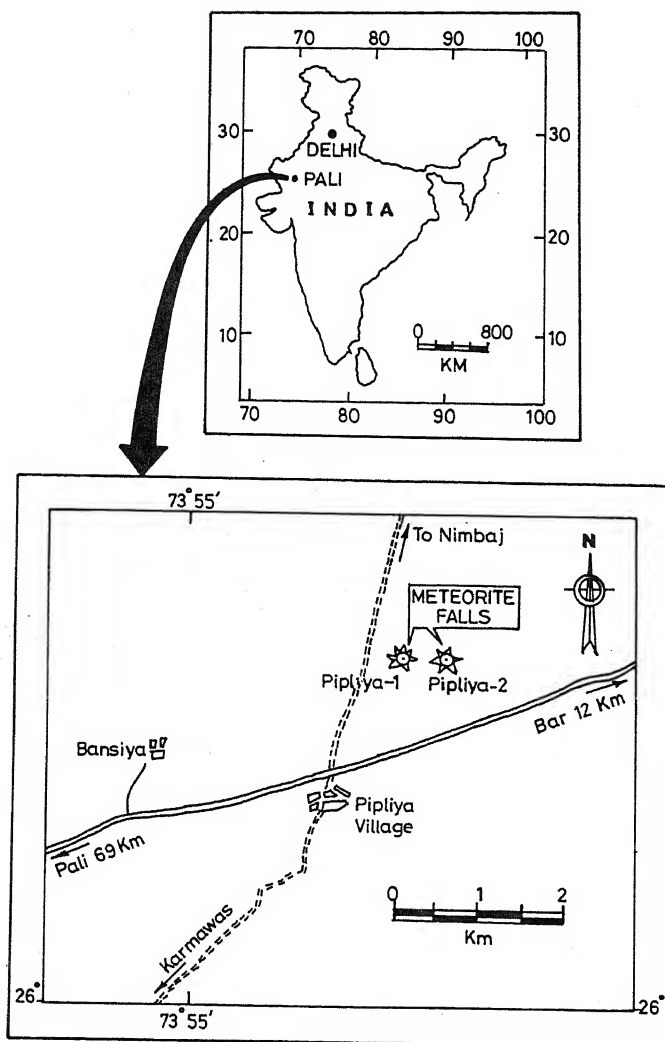


Figure 1. Location map: Meteorite fall on 20 June 1996 near village Pipliya Kalan, Dist. Pali, Rajasthan.

of the Pipliya meteorites is shown in Figure 1.

The two meteorites of the same meteor shower are hereby named as the Pipliya-1 and the Pipliya-2. The Pipliya-1 meteorite, approximately weighing 20 kg, was broken into many smaller pieces by Prabhu Lal Seervi. Nearly 15 kg of the pieces were recovered from him by the authorities of the Department of Mines and Geology, Government of Rajasthan. Another meteorite of the shower, weighing nearly 10 kg, was recovered by us 500 m away from the site of fall of the Pipliya-1. As meteorites could have a special distribution pattern from a meteor shower¹, we are intensively scaling nearly a 20 sq km area around Pipliya Kalan

following the local villagers' version that six fragments have fallen. This leaves four fragments yet to be recovered.

The crater formed due to the impact of Pipliya-1 is 50 cm deep and 70 cm in diameter. The splash reached up to 5 m away from the crater. There was no visible sign of scorching on a 10 m high tree, three metres away from the crater. The crater formed due to the impact of the Pipliya-2 is 40 cm deep and 50 cm in diameter. Both the craters are very small in size compared to the size of the meteorites. The cultivated soil in which craters were formed was wet and muddy due to rains. The meteorites and the craters are shown in Figure 2.

The Pipliya-2 has a glossy pitch black crust of nearly 500 μm thickness. The same type of crust is seen on different pieces of the Pipliya-1. The crust has no fissures but has thumb marks and knobs (Figure 2a) known in the meteorites². Megascopically, both the Pipliya-1 and the Pipliya-2 under the fused crust, are typical stony meteorites, gray to whitish gray in colour, coarse to fine grained in texture, friable and highly shattered in nature. Megascopically, both consist of two types of rocks welded together, hereafter referred to as type A and type B. Type A is a fine-grained gray coloured rock while type B is a coarse-grained, grayish white coloured rock with a very thin black welded boundary between the two types. Type A and type B are distinct microscopically both under ordinary light (Figure 3a) and crossed nicols (Figure 3b). The rock as a whole is a complex breccia consisting of fragments which are generally angular but sometimes rounded. The boundary of the fragments is dark gray in colour.

The study of thin sections under petrological microscope reveals that type A is composed of grains of pyroxene (65%), plagioclase (25%), opaque minerals (6%) and some xenoliths (4%). Type B is composed of pyroxenes (55%), plagioclase (40%) and opaque minerals (5%). Pyroxene is mostly clinopyroxene of pale pink colour, non-pleochroic, with moderate extinction angles. Some grains show twinning (Figure 3d) while some others show banding which may be exsolution lamellae. Plagioclase occur as euhedral to subhedral laths which are more elongated in type B. It is mostly bytownite as determined by Michel-Levy's method. Twinning is very complex in some grains. It shows twinning according to Carlsbad law either alone or combined with albite twinning and pericline twinning (Figure 3d). Opaque minerals could be magnetite as triangular, square or rhomb shaped sections are present which could be yielded by octahedral crystals of magnetite. Thin edges of these grains are neither brown or translucent nor white or yellow, which rules out the possibility that they may be chromite and ilmenite, respectively. (Note the limitations of the thin section study.) Type B shows equigranular and intergranular textures (Figure 3c), with minerals showing extreme shock effects:

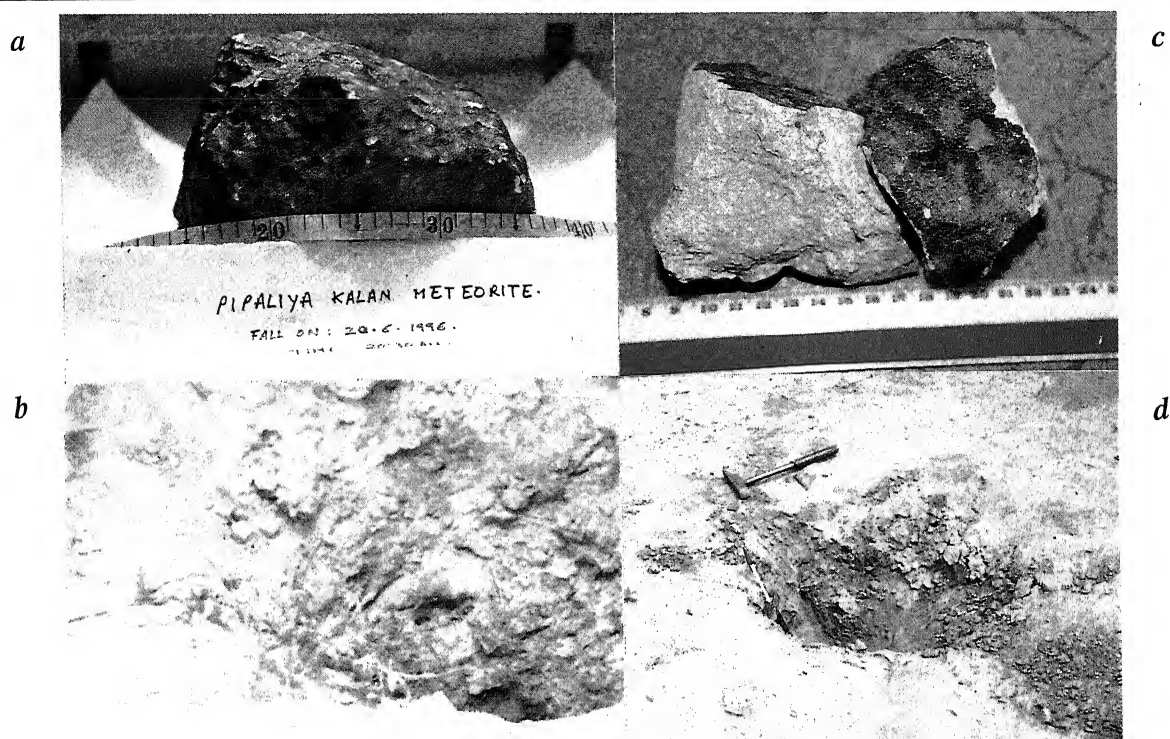


Figure 2 *a-d*. *a*, The Pipliya-2 meteorite; *b*, Crater formed by the Pipliya-2 meteorite; *c*, Pieces of the Pipliya-1 meteorite; *d*, Crater formed by the Pipliya-1 meteorite.

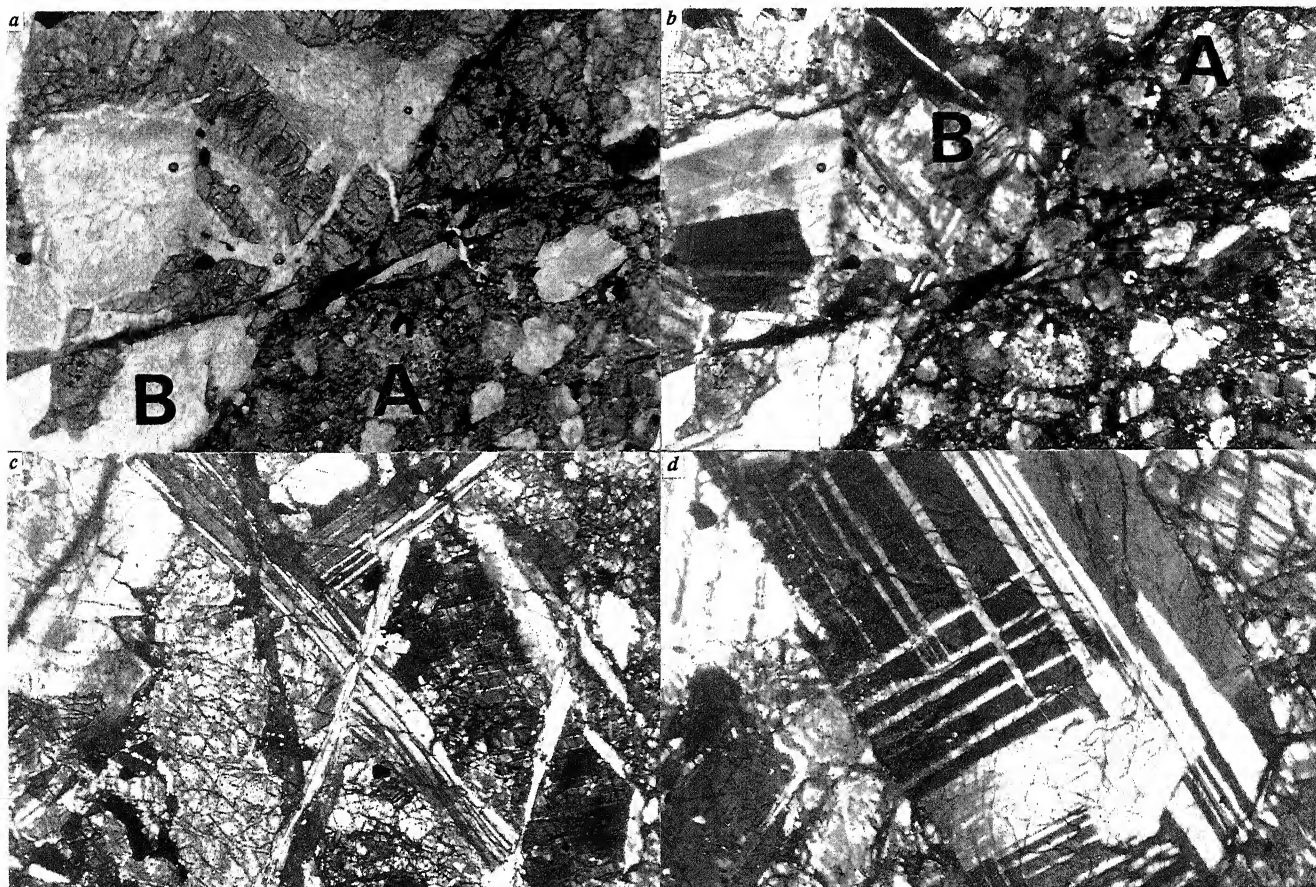


Figure 3 *a,b*. The Pipliya-1 meteorite under microscope showing type A and type B rock types (also indicated as A and B). The black line demarcates the boundary between the two. Pyroxene inclusions are present in type B. *a*, Ordinary light $\times 40$; *b*, Nicols crossed $\times 40$; *c*, Type B rock under microscope showing intergranular texture. Note fractures and displaced lamellae in plagioclase laths. (Nicols crossed $\times 40$); *d*, Plagioclase crystal showing twinning; under Carlsbad law, albite twinning and pericline twinning. (Nicols crossed $\times 100$).

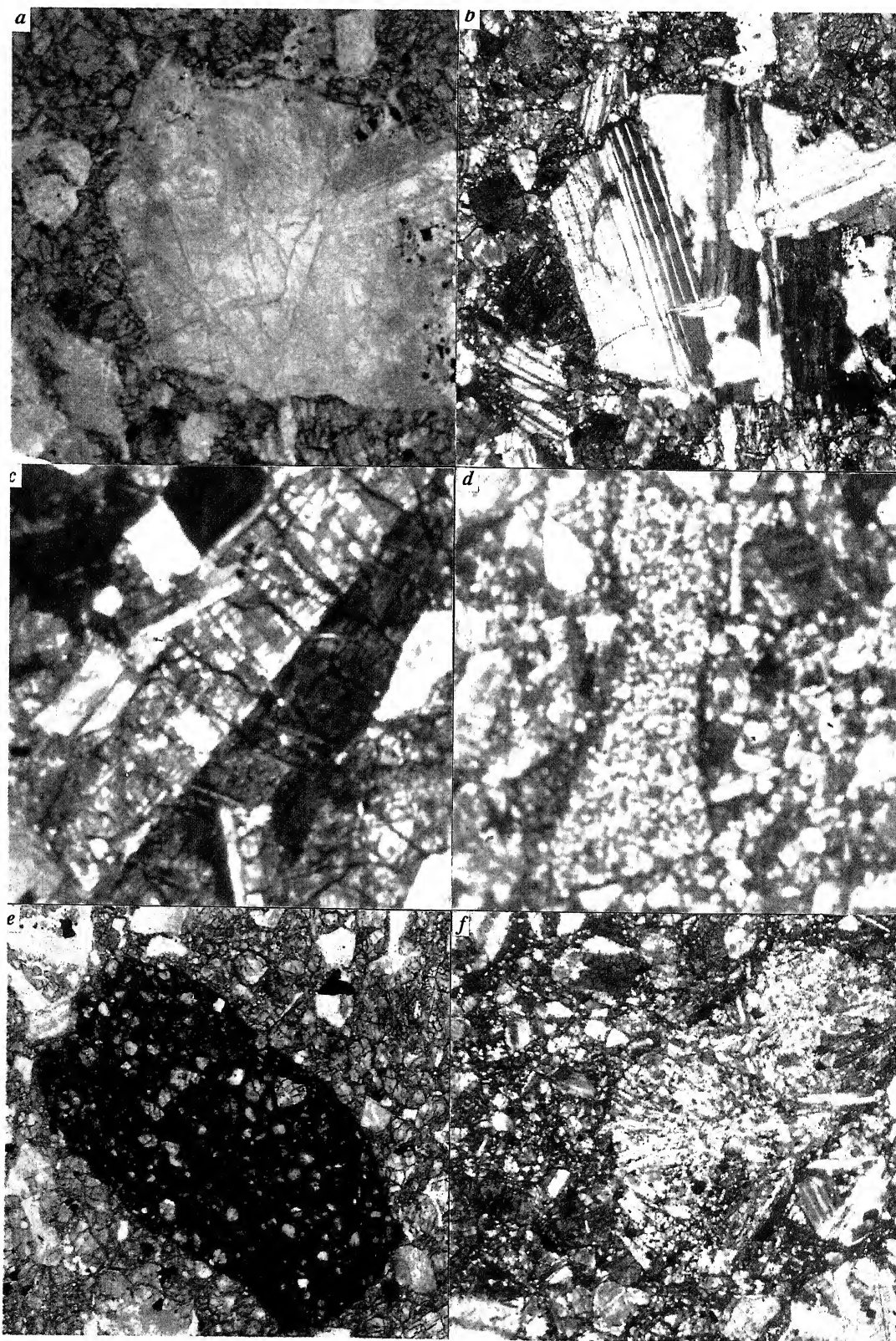


Figure 4 *a, b*. A plagioclase crystal showing prominent shock features under polarized light (*a*), and under crossed nicols (*b*) (both $\times 40$); *c*, A pyroxene crystal showing twinning under Carlsbad law. (Nicols crossed $\times 40$). *d*, Xenolith in type A, showing microcrystalline, equigranular texture. (Nicols crossed $\times 40$); *e*, Xenolith consisting of opaque mass showing pyroxene inclusions. (Nicols crossed $\times 40$); *f*, Xenolith showing granular pyroxene filling the spaces between the plagioclase laths. (Nicols crossed $\times 40$).

strain shadows, shattering, cloudy appearance, fractured and displaced lamellae, undulose extinction, etc. (Figures 3c, d and 4b). Type A shows subophitic to ophitic texture. Type A rock also shows some other fragments looking like xenoliths. One xenolith shows microcrystalline equigranular structure (Figure 4d), another encloses few pyroxene grains in a thick opaque background (Figure 4e). The third xenolith consists of granular pyroxene filling the

spaces between the rosettes of laths of plagioclase (Figure 4f).

The specific gravity of one of the pieces of the Pipliya-1 meteorite is 2.78 (mercury displacement method). There is no perceptible radioactivity as detected by scintillometer in any fragment of the meteorites.

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The expanding world of 2-acetyl-1-pyrroline

2-Acetyl-1-pyrroline (2AP) was first reported in 1982 as the principal component of the pleasant aroma of fragrant varieties of rice¹. Such a common source of fragrance was discovered so late, probably because of the unstable nature of the molecule and the general fact that even the comparatively blunt human nose is more sensitive than the sophisticated instruments of analytical chemistry.

Unexpected newer sources of 2AP are now being revealed. I first followed the trail of this elusive molecule (Figure 1) thirty-odd years ago when George Schaller had undertaken the first long-term study of the tiger and brought to the notice of scientists the musky, milky fluid sprayed upwards by tigers and tigresses, presumably to leave communicatory signals². With Schaller I noticed at Kanha, Central India, the smelly signal of a tiger, uncontaminated by the polluting odour of unclean zoos. In 1976 I had the opportunity of closely studying the fluid of a pet tigress and the odour of fresh sprayings seemed to be surprisingly similar to the aroma of

basmati and other fragrant rice (Figures 2, 3). Since then my colleagues and I have been studying the tiger fluid and we have established that the volatile smelly molecules are a number of amines, aldehydes, free fatty acids, etc., but the most elusive of them is a molecule similar to 2AP (refs. 3-7). Rice aroma and tiger aroma turned out to be identical in the course of investigations utilizing paper chromatography and gas-liquid chromatography but for lack of 'sniff GLC' (where one part of the test material is led to the flame ionization detector and the other, to the nose for sniffing) the latter set of experiments was not conclusive. Further confirma-

tion has now been obtained as described below.

Synthesis

Following the initial attempt of Buttery *et al.*¹, Schieberle⁸ succeeded in finding a simple method for synthesizing 2AP. Proline and a sugar (sucrose, fructose or glucose) form 2AP at 170°C. In our hands the procedure led to the production of two different fragrant substances S_1 and S_2 . S_2 seemed to be identical to the tiger aroma and rice aroma⁷. At first we had the impression that S_1 , the major component, was 2AP (ref. 7) but recently by

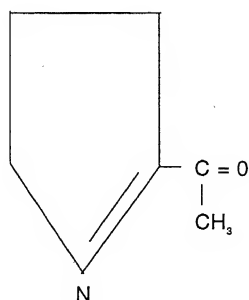


Figure 1. Structure of 2AP.



Figure 2. The raised tail of a tiger indicates that it is ready to spray a fluid containing 2AP.



Figure 3. Rice – A source of 2AP.

comparing authentic synthetic 2AP from Schieberle's laboratory we have established that S_2 of our first synthesis, rice aroma, tiger aroma and 2AP are identical as established by paper chromatography and GLC (squalane column).

In another synthesis we succeeded in obtaining a large amount of S_2 (2AP) but under the anhydrous conditions of Schieberle⁸ we usually obtained variable results. A slight modification using an aqueous solution of proline and sugar led to more uniform production of 2AP (ref. 9). The temperature of the solution inside the container was later measured and found to be between 105°C and 110°C. The temperature range for 2AP production is narrow; 2AP is not formed even at 103°C, but at 105°C the first whiff of the pleasant aroma is unmistakable.

As the quality of aroma in compounds like carvone and limonene is known to depend on the chirality of the molecule, we synthesized 2AP with both L-proline and D-proline. The quality of 2AP-aroma turned out to be independent of the chirality of proline.

Sources of '2AP-like aroma'

Fragrant rice, tiger and leopard produce this aroma which is absent in the African lion and almost absent in a cheetah I studied in Namibia. People in both England and India have described rice aroma as mousey. It is worth investigating whether certain strains of mice at certain times produce this odour. I have also smelt this fragrance in a certain breed of dog and, rarely, in human

sweat. A bintaurong in a clean zoo, at night, spread a very sweet odour which seemed to me to be exactly like that of the freshest, best-quality fragrant rice. It is to be noted, however, that civetone, which has a different chemical structure smells like 2AP as asserted by many people.

The fresh flowers of *Bassia latifolia* emit a smell which resembles the fragrance of rice and has also been described as mousey by a European. Paper-chromatography of this flower extract and synthetic 2AP suggest that the flower aroma is 2AP (ref. 10). Further work with GLC and HPLC may establish the contention and in that case this may be the first example of 2AP traced in the scent of flower.

Biosynthesis

The biosynthesis of 2AP is not clear. It may be enzymatic or, simply, a Maillard reaction, a principle described about 80 years ago but which is now evoking renewed interest¹¹. A Maillard reaction involving an amino-acid and a sugar may yield numerous products at very many different temperatures. In our attempts the lowest temperature that yields 2AP is 105°C, but in the rice plant this process occurs in the temperature gained from autumn sunshine (~30°C) and in *Bassia* flower, at dawn or evening or night in the spring season and therefore, at a much lower temperature. In some export-quality basmati rice, the sweet 2AP smell is not present in the unboiled grain. Boiling rice leads to further Maillard reaction and production of 2AP.

Unstable molecule

2AP decays very quickly, a fact that may explain why most urban people have no concept of the excessively sweet smell of freshly husked rice grains of many indigenous fragrant varieties. This smell disappears very quickly but even relatively old rice, on boiling, produces 2AP once again, presumably due to Maillard reaction. Buttery *et al.*¹ succeeded in keeping the synthetic molecule (as a hydrochloride) intact for 3 months. We note that the picrate is stable even up to 2 years. In a recent experiment, we found good stability as a citrate salt after 6 months and we hope to test again after one year of preservation.

Utility of 2AP for the plant

We enjoy the aroma of 2AP and probably the tiger uses it as a component of its pheromonal, communicatory signal but what good does this perfume do to the rice plant itself? This, at present, is anybody's guess but recently jasmonic acid and methyl jasmonate, the fragrant molecules of jasmine have been found to be useful as arsenals of the plant for fighting bacterial, viral and fungal attack¹². A fragrant rice variety in West Bengal has recently been noticed to be unusually fungus-resistant¹³. The role of 2AP, if any, in protecting the rice plant may be studied in future.

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Somatic embryogenesis and plant regeneration in *Aegle marmelos* – a multipurpose social tree

Aegle marmelos Corr. (Rutaceae) locally called 'Beal' is an economically important multipurpose social tree of the Indian subcontinent. Micropropagation of necessary useful trees enables rapid propagation and hastens the availability of new cultivars¹. Regeneration from hypocotyl², cotyledon³, leaf⁴, nucellus^{5,6} and zygotic embryos⁷ was achieved. However, plant regeneration via somatic embryogenesis has not yet been reported in *A. marmelos*. Somatic embryogenesis has been achieved in a number of angiosperms but success has been limited with woody species^{8–11}. Here we report somatic embryogenesis of *A. marmelos* by using zygotic embryos.

Seeds from mature, green unripe fruits of 20-year-old *A. marmelos* were surface disinfected with 0.1% mercuric chloride. Embryo axis containing one-fourth part of cotyledons was excised from decoated seeds and cultured on solid Murashige and Skoog (MS) medium¹² containing 20% coconut water, 4% sucrose, 2,4-dichlorophenoxyacetic acid (2,4-D) and benzyladenine (BA) at different concentrations (0.5–2.0 μM) and combinations. After six weeks the somatic embryos were transferred into test tubes containing half strength MS medium supplemented with 2% sucrose and 1.0 μM BA. The cultures were incubated at $26 \pm 1^\circ\text{C}$ with 16 h photope-

riod (50–70 $\mu\text{mol m}^{-2} \text{s}^{-1}$, Phillips white fluorescent lamps).

White globular somatic embryos appeared as loose structures after 30 days on explants (Figure 1a). The highest response in number of explants producing somatic embryos (18%) and the number of somatic embryos per explant (12) was observed in presence of 2,4-D (1 μM) and BA (1 μM) as per observations recorded after 42 days. Media supplemented with 2,4-D alone did not produce any somatic embryos. In combination with BA (0.5–2.0 μM), 2,4-D at greater than 1.00 μM also showed inhibitory effects on the induction of somatic embryos.

After six weeks of culture initiation, somatic embryos were transferred to half-strength MS medium with 2% sucrose and 1.0 μM BA, radicals and apices of the somatic embryos became active within 15 days, cotyledons turned dark green and shoots elongated. This was consistent with observations that somatic embryos of woody species benefit from a reduction in nutrients in the culture medium for germination^{13,14}. More often, calli rather than root, developed at the radicle ends of the somatic embryos (Figure 1b). The conversion percentage was approximately 12% (Figure 1c). Culture conditions such as growth-regulator concentration and incubation time may affect germination and conversion into plants.

This is the first report on somatic embryogenesis in *A. marmelos*. Similar reports on *Liriodendron*¹⁵, *mango*¹⁶ and *lyohee*¹⁷ have appeared earlier. However, somatic embryogenesis in the present studies could prove useful in improving a multipurpose tree, *A. marmelos*.

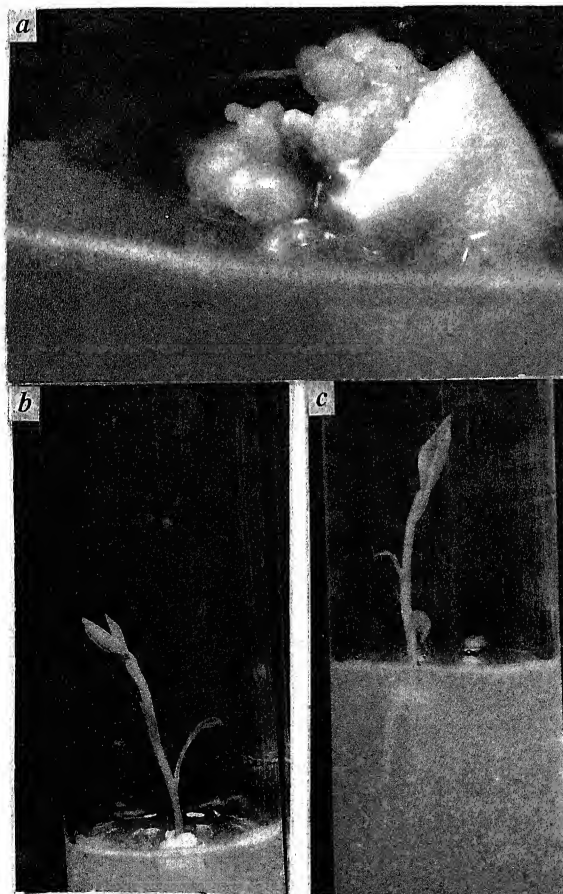


Figure 1 a–c. Somatic embryogenesis in *A. marmelos*. a, Formation of globular somatic embryos on zygotic embryo in MS + 1.0 μM 2,4-D + 1.0 μM BA after 5 weeks of culture. b, Somatic embryo developed into shoot on 1/2 MS + 1.0 μM BA after 5 weeks of culture; note formation of callus on radicle portion. c, A complete plantlet developed in the same medium.

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Norbert Wiener and the development of mathematical engineering

Thomas Kailath

1. Introduction

'There is nothing better than concrete cases for the morale of a mathematician. Some of these cases are to be found in mathematical physics and the closely related mathematical engineering...'

So wrote Norbert Wiener in 1949, in an obituary of G. H. Hardy, who reputedly would have shuddered at the thought. [Ironically, Hardy's own major field of number theory has been of great importance for many applications, for example in secure data communications.] This paper will attempt to describe how one particular concrete problem in Wiener's own work – solving the Wiener–Hopf equations encountered in astrophysics – led him, and then a vast host of followers, to chart out several new areas of investigation, and to develop a very significant body of knowledge, which can well go by the name Mathematical Engineering. In the era of the PC, the Internet and the World Wide Web, few of us can be unaware that mathematical engineering has come to play a major role in the world around us. And with this has come, as this paper will describe, an increasing recognition of the seminal role of Norbert Wiener's ideas and influence in the development of this field.

It must be said that the term 'Mathematical Engineering' does not enjoy the currency that the name 'Mathematical Physics' does. Being a younger field, its proponents still focus on more specialized descriptions such as Information Theory, Communications, Control, Signal Processing, Computational Complexity, Image Processing, etc. The names 'System Theory' or even 'Mathematical System Theory' have been advanced but are not universally accepted. However this author believes that the increasing intermingling of the fields mentioned above, with many tools and techniques being successfully applied across them, as well as the tremen-

dous opportunities ahead of them in the Information Era, could well lead to the adoption of Wiener's suggestion. And be that as it may, Wiener's early vision and pioneering contributions will, as mentioned earlier, loom even larger with time. Already in 1962, in a special issue commemorating the 50th anniversary of the effective existence of the IEEE (Institute of Electrical and Electronics Engineers), Lotfi Zadeh, winner of the 1995 IEEE Medal of Honour wrote *'If one were asked to name a single individual who above anyone else is responsible for the conception of system theory, the answer would undoubtedly be 'Norbert Wiener', even though Wiener has not concerned himself with system theory as such, nor has he been using the term "system theory" in the sense employed in this paper'*.

There are many of Wiener's results that have come to be important in mathematical engineering. Although Wiener was apparently never quite secure about his place in the pantheon of scientific innovators, he seemed not to have such doubts about the significance of the particular concrete problem that we shall concentrate on in this paper. This is the Wiener–Hopf equation, which Wiener first encountered in 1931 in astrophysics and then a decade later in the problem of anti-aircraft fire control. I hope to indicate how Wiener's work on this equation has led to the development of a remarkably broad, and deep, range of studies.

In the next section, we shall describe Wiener's beautiful technique of spectral factorization for solving it. In § 3 and 4, we shall note that Wiener encountered the equation again in solving a problem in anti-aircraft fire control and how his 1942 report on this project introduced two fundamental ideas that radically changed the way engineers approached important classes of problems. First is that the communication of information must be formulated as a statistical problem; the second, the introduction of optimization criteria to obtain the limits of performance and replace the earlier 'trial and error' approach to design. From these two ideas has grown the huge flood of activity noted earlier. However to narrow the scope we shall return in the remainder of the paper to a specific problem studied by Wiener – filtering signals out of noisy observations. After describing his results, we shall turn to some of the mathematical developments following from it. First we shall show in

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§ 6, how, while Wiener was not quite successful in extending his results to the case of multiple time series, this can be done by the introduction of state-space and Markov process descriptions. Somehow Wiener himself never really focused on the Markov property. As we shall see, the state-space description introduces the concept of recursive solution algorithms and enables straight-forward extension to nonstationary/time-variant versions of the filtering problem. In § 7, we shall examine a widely used finite-time prediction problem, which has been generalized by using the concept of displacement structure. Finally in § 8, we shall introduce the nonlinear filtering problem, which is still open, but for which a key tool is martingale theory, the prototype for which was the Wiener (Brownian motion) stochastic process.

2. The Wiener-Hopf equation

Given $\phi_{11}(\cdot)$ and $\phi_{12}(\cdot) \in \mathcal{L}_1(\infty, -\infty)$, $\phi_{11}(\cdot)$ even and positive definite, find $k(\cdot)$ such that

$$\phi_{12}(t) = \int_0^\infty \phi_{11}(t-\tau)k(\tau)d\tau, \quad t \geq 0 \quad (1)$$

where $k(t) = 0$, $t < 0$. Equations of this type were apparently first introduced by Hvol'son (1894, Leningrad) while studying the scattering of light by milk glass, and later (ca 1920), by E. A. Milne, K. Schwarzschild and others in problems in astrophysics.

While such equations attracted considerable attention, explicit analytical solution was long thought to be impossible, a point of view that, in the words of M. G. Krein (1958) 'was refuted in 1931 by the brilliant achievement of E. Hopf and N. Wiener'. In fact, so striking in its ingenuity and simplicity was their solution that not only the technique, but the equation itself came to be known by the name 'Wiener-Hopf'. Moreover a decade later, Wiener encountered the equation again, with even more significant consequences!

The genesis of these events was apparently (Pincus (1981)) a summer evening with E. Hopf at Wiener's country home in New Hampshire, where characteristically Wiener asked his visitor about challenging open problems in his field. The next morning Wiener came down for breakfast with a 'solution'. It in fact needed some reworking, which was done and the result was published in 1931, with the unusual (for mathematicians) nonalphabetical ordering of names. We shall briefly outline the Wiener-Hopf idea (ignoring various technical assumptions, for which see, e.g., Krein (1958)).

The tools used in the solution had in fact largely been developed by Wiener himself in collaboration with R. E. A. C. Paley. First were the properties in the complex plane of Fourier transforms of one-sided (or 'causal')

functions. One motivation for Wiener's interest in such topics may have been his study of electrical networks and his 1931 patent (with Y. W. Lee, Sc. D., Elec. Engg.) on a new cascade realization (based on Laguerre functions), of network impedance functions, known to mathematicians as Caratheodory (or positive-real) functions. In any case, for us the key property is that (again under certain conditions) a function $k(\cdot)$ such that $k(t) = 0$, $t < 0$, has a Fourier transform $\mathcal{K}(\omega)$ whose extension to the complex plane, $\mathcal{K}(s)$, $s = \sigma + i\omega$, is analytic in the RHP, $\sigma > 0$.

The second tool was the closely related fact that such extensions of nonnegative even functions

$$0 \leq \Phi(\omega) = \Phi(-\omega) \quad -\infty < \omega < \infty$$

have unique canonical factorizations

$$\Phi(s) = \Psi(s)\Psi^*(-s^*), \quad s = \sigma + i\omega \quad (2)$$

where $\Psi(s)$ is analytic and bounded in the RHP, $\sigma > 0$, while $\Psi^{-1}(s)$ is analytic in the RHP, $\sigma > 0$. It may be noted that Wiener had recognized such functions, $\Phi(\cdot)$, as Fourier transforms of autocorrelation (real, even and positive definite) functions and described their significance as power spectral density functions. An equivalent result was discovered by Khinchin in 1934, leading to the so-called famous Wiener-Khinchin theorem. [It turns out that they had been anticipated by no less than Albert Einstein, in a two-page 1914 sketch answering a question raised by a friend on measuring the 'power' of meteorological time-series!] Now for the Wiener-Hopf technique.

We first extend the equation (1) to the whole line by introducing the function

$$g(t) = \phi_{12}(t) - \int_0^\infty k(\tau)\phi_{11}(t-\tau)d\tau \quad -\infty < t < \infty.$$

By the fact that (1) only holds for $t \geq 0$, we know that $g(\cdot)$ is one-sided, but 'anticausal',

$$g(t) = 0 \quad t \geq 0$$

unlike the unknown 'causal' function $k(\cdot)$,

$$k(t) = 0 \quad t < 0.$$

Of course now we have two unknown functions, $g(\cdot)$ and $k(\cdot)$, but give us a moment. Take Fourier transforms in the complex plane to get

$$G(s) = \Phi_{12}(s) - \mathcal{K}(s)\Phi_{11}(s).$$

Now use the canonical factorization (2) to write

$$\frac{G(s)}{\Psi^*(-s^*)} = \frac{\Phi_{12}(s)}{\Psi^*(-s^*)} - \mathcal{K}(s)\Psi(s)$$

But by construction $G(s)$ and $1/\Psi^*(-s^*)$ are analytic in the LHP, $\sigma < 0$, while $\mathcal{K}(s)$ and $\Psi(s)$ are analytic in the RHP, $\sigma > 0$. Equivalently the time function obtained by

inverse Fourier transformation (IFT) of $G(s)/\Psi^*(-s^*)$ will be zero for $t \geq 0$ (anticausal), while the time function corresponding to $\mathcal{K}(s)\Psi(s)$ will be zero for $t < 0$ (causal). This means that the latter time function must be equal to the $t \geq 0$ portion of the IFT of $\Phi_{12}(s)/\Psi^*(-s^*)$, leading to the famous formula for the solution of the Wiener-Hopf equation (1)

$$\mathcal{K}(s) = \frac{1}{\Psi(s)} \int_0^\infty dt e^{-st} \oint \frac{\Phi_{12}(p)}{\Psi^*(-p^*)} e^{pt} \frac{dp}{2\pi i}. \quad (3)$$

We shall return to this equation in § 5, after an apparent digression.

3. The problem of anti-aircraft fire control

Seeking eagerly to contribute to the War effort, Wiener submitted a proposal to the National Defense Research Council (NDRC) for a novel parallel computing machine for solving partial differential equations, as arising for example in aerofoil design problems. However, the proposal was deemed unlikely to be completed in a reasonable time frame. Wiener cast about for a more relevant problem and hit upon the problem of anti-aircraft fire control. In Dec. 1940, he sought and won a (\$2350!) project on '*design of a lead or prediction apparatus in which, when one member follows the track of an airplane, another anticipates where the airplane is to be after a fixed lapse of time*'. Under certain assumptions, the problem can be reduced to one of finding an effective method of approximating an exponential function by a rational function of suitable order, which Wiener proposed to do by using ideas from his earlier (ca 1930) work with Y. W. Lee on network synthesis. However working with the engineer hired for the project, Julian Bigelow, Wiener came to see in a few months, that because of noise and of model uncertainties, a satisfactory engineering solution demanded explicit consideration of the *statistical nature* of the problem, and also the use of an *optimization criterion*. Effectively assuming that the airplane trajectories were sample functions of a stationary and ergodic random process, and imposing the requirements of a *linear apparatus* and of the *mean-square-error criterion* led him again to the Wiener-Hopf integral equation. So the solution was at hand! But unlike the apocryphal mathematician, who promptly drops a problem when he reduces it to one that has already been solved, Wiener realized that much more was needed for a real engineering solution.

In fact, Wiener's project reports make a beautiful (and perhaps the first) case study in how to use the theory of *optimal* solutions in the real world. First, he confined himself to the practically most significant case: rational power spectral densities, necessary for '*the actual realization in the field by a finite electrical or mechanical structure*'. Here are a few other quotations: '*the detailed*

design of a filter involves ... choices ... which must be justified economically'. '*Let us see ... the stages that are needed. The first stage determines the irreducible error, i.e., the error which cannot be reduced by any delay whatever. ... Next, ... , we can determine a reasonable delay, such that the delay error is not large in comparison with the irreducible error, the sense of 'large' depending on the problem.*' Writing about the assumption of stationarity, he reassures the user that while the available '*statistical information will in fact never be complete, as our information does not run indefinitely for back into the past, it is a legitimate simplification of the facts to assume that the available information runs back much farther into the past than we are called upon to predict the future*'. And so on. Few mathematicians before Wiener had such an interest in the many issues arising in actual implementation.

Despite all this, however, the results actually obtained in field trials were not satisfactory, and the project was terminated. But Wiener wrote up the theoretical results obtained in the course of the work, along with some mathematical preliminaries and his philosophical views on the nature of his new approach to the field of communication engineering, in a remarkable 1942 monograph '*Extrapolation, Interpolation and Smoothing of Stationary Time Series*'. It was later declassified and published in 1949 by J. Wiley and the MIT Press. The classified report was widely circulated, while the engineers of the day were hard pressed to follow its contents, it was closely studied by mathematicians such as N. Levinson and R. Philips at MIT, and H. W. Bode, C. E. Shannon, R. Blackman and others at Bell Laboratories. They all wrote up expositions and variations of Wiener's results, some of which we shall mention later.

While preparing his report, Wiener was made aware (by W. Feller) that some-what earlier, and apparently as a purely mathematical investigation, the famous Soviet mathematician A. N. Kolmogorov had studied the prediction problem for general discrete-time stationary processes. The approaches were quite different: Wiener's concrete and focused on applications, Kolmogorov's more abstract and more general. Though later both the methodologies – concrete and abstract – became useful in applications, it was Wiener's work that had a greater impact on electrical engineering, far beyond the original problem and solution.

4. Two new paradigms

In fact, Wiener was quite aware (and perhaps too much so) of the revolutionary significance of his work and ideas, and he stated his views quite emphatically in his 1942 monograph and in various other talks and papers: several passages from these works are quoted here, to show the clarity and prescience of Wiener's thinking.

We begin with his clear and forceful statement of the **statistical nature of the communication problem**. Thus on p. 2 of his monograph:

'Communication engineering concerns itself with the transmission of messages. For the existence of a message, it is indeed essential that variable information be transmitted. The transmission of a single fixed item of information is of no communicative value. We must have a repertory of possible messages, and over this repertory a measure determining the probability of these messages.'

'A message need not be the result of a conscious human effort for the transmission of ideas. For example the records of current and voltage kept on the instruments of an automatic substation are as truly messages as a telephone conversation.'

And then on p. 4:

'A statistical method, as for example a method of extrapolating a time series into the future is judged by the probability with which it will yield an answer correct within certain bounds, or by the mean (taken with respect to probability) of some point, i.e., function or norm of the error contained in its answer.'

'No apparatus for conveying information is useful unless it is designed to operate, not on a particular message, but on a set of messages, and its effectiveness is to be judged on the way performs on the average on messages of this set. ... The apparatus to be used for a particular purpose is that which gives the best result 'on the average' in an appropriate sense of the word 'average'.'

To those now familiar with Information Theory, presented by Shannon in 1948, and Signal Detection Theory as presented by P. M. Woodward and others in the early 1950s, it is worth emphasizing that the above passages were written in 1942. As one major illustration of their influence, we may note Shannon's gracious acknowledgement at the end of his magnificent 1948 paper founding Information Theory:

'Credit must also be given to Professor Norbert Wiener, whose elegant solution of the problems of prediction and filtering has considerably influenced the writer's thinking in this field.'

But there is more. Going beyond the statistical problems he had studied, Wiener stressed the possibility of formulating a variety of engineering problems as *optimization* problems, so that *performance limits* may be calculated and *reasonable* solutions sought; this stands in sharp contrast to many earlier *trial and error* methods:

'These specifications give us an optimum filter to fit the situation exactly, whereas the earlier methods designed filters to certain specifications concerning

passbands, sharpness of cut-off, etc., which stood in no obvious relation to the actual demands of a problem and had to be adjusted to these by the process of trial and error [N. Y. Acad. Sci. lecture, Oct. 1946].'

and again from his 1942 monograph:

'Prediction and filtering do not exhaust the capacity of our methods. They may be applied whenever an ideally desirable linear operation ... is in fact not strictly realizable, although an approximation may be realized.'

In particular, Wiener suggested that his ideas could be applied to the design of compensators for control systems. This suggestion was picked up in the Ph D research of R. Newton at MIT, who, along with L. Gould and J. Kaiser, wrote a book on the topic entitled *Analytical Design of Linear Feedback Controls*, Wiley, 1957. From it, we may quote passages that clearly echo Wiener's early insights (in the fifties, authors were not as gender conscious as they are now):

'... The analytical design procedure has several advantages over the trial and error method, the most important of which is the facility to detect immediately and surely an inconsistent set of specifications. The designer obtains a 'yes' or 'no' answer to the question of whether it is possible to fulfill any given set of specifications; he is not left with the haunting thought that if he had tried this or that form of compensation he might have been able to meet the specifications.'

'... Even if the reader never employs the analytical procedure directly, the insight that it gives him into linear system design materially assists him in employing the trial and error design procedure.'

However more than 50 years later, Wiener's message is apparently still worthy of repetition. Thus, let us quote from a very recent text book by M. Green and D. Limebeer, *Linear Robust Control*, Prentice-Hall, 1995:

'One does not want to waste time trying to solve a problem that has no solution, nor does one want to accept specification compromises without knowing that these are necessary. A further benefit of optimization is that it provides an absolute scale of merit against which any design can be measured – if a design is already all but perfect, there is little point in trying to improve it further.'

'The aim of this book is to develop a theoretical framework within which one may address complex design problems with demanding specifications in a systematic way.'

We continue with some further remarks from this book on the Wiener ideas, though the authors here cite

also the work of R. E. Kalman, which we shall discuss in a later section. From p. 2, we quote (note the 'free ride' that Hopf gets; he perhaps was completely unaware of these applications):

Wiener-Hopf-Kalman optimal control

'The first successes with control system optimization came in the 1950s with the introduction of the Wiener-Hopf-Kalman (WHK) theory of optimal control. At roughly the same time the United States and the Soviet Union were funding a massive research program into the guidance and maneuvering of space vehicles. As it turned out, the then new optimal control theory was well suited to many of the control problems that arose from the space program.'

'... [A] revolutionary feature of the WHK theory is that it offers a true synthesis procedure. Once the designer has settled on a quadratic performance index to be minimized, the WHK procedure supplies the (unique) optimal controller without any further intervention from the designer.'

However, the major motivation for Green and Limebeer's 1995 book was the fact that they were introducing a new (so-called H_∞) formulation:

'In contrast to experience with aerospace applications, it soon became apparent that there was a serious mismatch between the underlying assumptions of the WHK theory and industrial control problems. Accurate models are not routinely available and most industrial plant engineers have no idea as to the statistical nature of the external disturbances impinging on their plant'

Worst-case control: H_∞ optimization

' H_∞ optimal control is a frequency-domain optimization and synthesis theory that was developed in response to the need for a synthesis procedure that explicitly addresses questions of modeling errors.'

Ironically, the new H_∞ theory can be regarded as Wiener-Kalman theory, but in Krein space rather than in Hilbert space. More on this later.

Here, however, as a final quotation from Wiener's own monograph, let us present one that illustrates Wiener's keen insight into what mathematical refinements are relevant to engineers. Engineering textbooks are often still concerned about the level of rigor at which to present Fourier Theory – should one worry about pointwise convergence, L_2 convergence, etc? Wiener cuts right to the heart of the matter:

'Up to the present we have been treating the Fourier series as a purely formal expression without any regard to whether it converges or not. ... Now it is obvious that no physical quantity can be observed for a single precise value of t Thus all functions of t are

for the physicist averages over small ranges of t rather values of a precise point of t .' [He goes on to very simply show that] *'all local averages of the formal [Fourier] series [of $f(t)$] converge to the local average of $f(t)$. As we have pointed out, this is all that we need to make a practical employment of the Fourier series for $f(t)$.'*

This is the approach that later in 1948 L. Schwartz elaborated in his theory of distributions which, among many other things, made rigorous the widespread use of impulsive functions by engineers. I cannot resist remarking that Wiener also uses the 'Sampling Theorem for Bandlimited Functions', first derived by J. M. Whittaker in 1915, but to this day widely cited as Shannon's Sampling Theorem. There are several other little gems in Wiener's report, now fortunately also available in an MIT Press paperback entitled 'Time Series'. But let us now return to some more explicit mathematical problems.

5. The Wiener filter

The appearance in 1949 of Wiener's monograph, and of Shannon's work on Information Theory, generated a huge wave of activity in, to use Wiener's phrase, 'mathematical engineering'. A striking range of mathematics has and is being used in these studies, as can be seen by glancing through, for example, the IEEE Transactions on Information Theory, on Automatic Control, on Signal Processing, on Circuits and Systems, on Image Processing, etc. Many of the algorithms were too complex for implementation till about five to ten years ago, but the pace of technology insertion is accelerating.

In the rest of this paper, we shall discuss one of the directions most closely related to Wiener's own work, focusing on some problems left open by him: spectral factorization of matrix-valued rational spectral densities; finite-time problems/nonstationary processes; nonlinear estimation/random signal detection. The idea is to give a glimpse of part of the wide range of consequences arising from just one of Wiener's mathematical contributions.

We begin with a review of the so-called (causal and noncausal) Wiener filters. A filter is a selective device, e.g., one that allows the transmission of certain frequency regions (the passband) and rejects certain other regions (the stop band). In filtering signals out of a combination of the signals and additive noise, the conventional solution was a device that rejects as much of the noise as possible while passing the signal through undistorted. If as is usually the case, the signal is band-limited (i.e., occupies a limited frequency range) while the noise is wideband, the solution is the so-called Ideal Filter, with unit gain in the frequency regions of the signal and zero gain elsewhere. Wiener pointed out that if

the signal is a random process, one might better be more concerned about regions where the power spectrum of the signal is much larger than that of the noise, with proper weighting to be assigned to different frequency regions depending upon the choice of an optimization criterion. The most fruitful has been Wiener's own choice of a least-squares criterion.

To be specific, consider the problem of estimation the value at a given time t of a stochastic signal process $s(\cdot)$ given the noisy observations

$$y(\tau) = s(\tau) + v(\tau) \quad -\infty < \tau < \infty \quad (4)$$

by using a linear time-invariant filter $h(\cdot)$ such that

$$\hat{s}(t) = \int_{-\infty}^{\infty} h(t-\tau)y(\tau)d\tau$$

satisfies (E denotes expectation)

$$E|s(t) - \hat{s}(t)|^2 = \text{minimum.}$$

Under the assumption that the processes $\{s(\cdot), v(\cdot)\}$ are zero mean and jointly stationary, it is not hard to check that the optimum filter must satisfy the equation

$$\phi_{sy}(t) = \int_{-\infty}^{\infty} h(t-\tau)\phi_{yy}(\tau)d\tau$$

where the $\phi(\cdot)$ are the autocorrelation functions

$$\phi_{sy}(t) = Es(\sigma+t)y^*(\sigma), \quad \phi_{yy}(t) = Ey(\sigma+t)y^*(\sigma).$$

This equation is easily solved by Fourier transformation to yield (using capitals for the transforms)

$$H(\omega) = \frac{\Phi_{sy}(\omega)}{\Phi_{yy}(\omega)}.$$

When the signal and noise processes are uncorrelated with each other, this reduces to

$$H(\omega) = \frac{\Phi_{ss}(\omega)}{\Phi_{ss}(\omega) + \Phi_{vv}(\omega)}. \quad (5)$$

To reflect the fact that the noise has a much higher frequency range than the signal, engineers often assume that the noise is *white*,

$$\Phi_{vv}(\omega) = R > 0, \quad -\infty < \omega < \infty. \quad (6)$$

Such noise processes of course fall out of the scope of the usual theory of stationary processes, since for one thing, the IFT of $\Phi_{vv}(\omega)$ is a delta function. And, in fact, since to have finite power all physical processes must have spectra that decay to zero as $\omega \rightarrow \infty$, white noise is nonphysical as well. Yet it is widely used, as a model, for several important (mathematical and physical) reasons that we do not have time to explain here. However, it is interesting to see Wiener's free use of the white noise model, which is important for many reasons, both theoretical and practical:

'As for (the noise), we shall take a case which, although not formally contained in the theory we have

given, constitutes the limiting case of it, and one of the greatest importance in practice. This is the case in which the noise input is due to a shot effect and has an equipartition of power in frequency. Theoretically, of course, this is not strictly realizable, as it would demand an infinite power; practically as in the case of Plank's law in optics, it may hold within the limits of observation up to frequencies of a magnitude so great that they are no longer of interest for our particular problem.'

To return to our problem, with the white noise assumption, we can see that

$$H(\omega) = \frac{\Phi_{ss}(\omega)}{\Phi_{ss}(\omega) + R} \rightarrow \frac{\Phi_{ss}(\omega)}{R} \text{ as } R \rightarrow \infty.$$

On the other hand, as $R \rightarrow 0$, $H(\omega)$ tends to the ideal filter (with unit gain in the passband of the signal), but this is not the optimum filter when $R \neq 0$. As $R \rightarrow \infty$, we note that $H(\omega) \rightarrow \Phi_{ss}(\omega)/R$, so that the filter tends to reinforce frequencies where $\Phi_{ss}(\omega)/R > 1$, and to suppress the signal in regions where $\Phi_{ss}(\omega)/R < 1$. This fits, in hindsight, with our intuition, but the theory is necessary to tell us what to do for arbitrary values of R , or for nonwhite (often called coloured) noise.

The above solution, though given by Wiener, does not use the Wiener-Hopf equation, because we are assuming that the process $y(\cdot)$ is observed over all time instants, past as well as future. When the observations of $y(\cdot)$ are restricted to the past, we have to solve a Wiener-Hopf equation, which when (4) and (6) hold, takes the form

$$Rk(t) + \int_0^{\infty} k(\tau)\phi_{ss}(t-\tau)d\tau = \phi_{ss}(t), \quad t \geq 0. \quad (7)$$

With the white noise assumption, the formula for the solution takes the striking form (apparently first noted by Yovits and Jackson (1960))

$$K(\omega) = 1 - R^{1/2}\Psi^{-1}(\omega). \quad (8)$$

In other words, the *canonical spectral factorization* completely defines the estimator in the additive white noise case!

One might justly wonder about the physical significance of the canonical factorization, and the stochastic problem allows a nice (and far reaching) interpretation, first given by Bode and Shannon (1950), and independently (and in somewhat more general form) in a lesser-known paper of Zadeh and Ragazzini, also appearing in 1950. These authors noted that passing the observations process $y(\cdot)$ through a linear filter with transfer function $\Psi^{-1}(\omega)$ gives us a process $e(\cdot)$ with power spectrum (using well-known formulas),

$$\Phi_{ee} = \Psi^{-1}(\omega)\Phi_{yy}(\omega)\Psi^{-*}(-\omega) = I.$$

Therefore, we can interpret the first term $\Psi^{-1}(\omega)$ in the general Wiener-Hopf formula (3)

$$k(\omega) = \frac{1}{\Psi(\omega)} \left[\int_0^\infty dt e^{-j\omega t} \oint \frac{\Phi_{12}(p)}{\Psi^*(-p^*)} e^{pt} \frac{dp}{2\pi i} \right],$$

as allowing us to replace the observations process $y(\cdot)$ by a much simpler stochastic process $e(\cdot)$, for which the problem of estimating a related stochastic process $s(\cdot)$ turns out to be much simpler: when $\phi_{11}(\cdot)$ in the Wiener-Hopf equation

$$\phi_{12}(t) = \int_0^\infty \phi_{11}(t-\tau) k(\tau) d\tau, \quad t \geq 0$$

is a delta function, the solution is immediate: $k(t) = \phi_{12}(t)$, $t \geq 0$.

One might wonder if there is a 'loss of information' in going from the original observed process $y(\cdot)$ to the white process $e(\cdot)$? The answer is no, because by the fact that the canonical factor $\Psi(s)$ and its inverse $\Psi^{-1}(s)$ have the property that they are analytic in the RHP, one can pass (recall the Paley-Wiener results quoted in § 3) from $y(\cdot)$ to $e(\cdot)$ and from $e(\cdot)$ back to $y(\cdot)$ by causal and stable linear operations. Since

$$\mathcal{F}^{-1}\{\Phi_{ee}(\omega)\} = Ee(\tau+t)e^*(\tau) = \delta(\tau) = 0, \quad \tau \neq 0$$

the value of $e(\cdot)$ at any instant is uncorrelated with its values at any other instant, and therefore every observation, $e(t)$, brings *new* information, which cannot be said about a (corrected) nonwhite process. The process $e(\cdot)$ is called the *innovations process* of $y(\cdot)$; we shall return to it in a more general context in § 8. The innovations concept has been useful in extensions of Wiener filtering theory to nonstationary processes and to nonlinear problems, see e.g., Kailath (1970), Davis (1977) and Fujisaki *et al.* (1971). We may note that, Kolmogorov's more general approach to the discrete-time prediction problem (1939), (1941) was based on the use of the innovations process, which avoids (or rather trivializes, as we noted earlier) the use of the Wiener-Hopf equation. Thus somewhat ironically, Kolmogorov's more abstract approach ultimately became more powerful than Wiener's more concrete approach, a phenomenon, mathematicians may be pleased to know, that is not uncommon in applications.

6. Extensions: Matrix spectral factorization

Wiener's monograph inspired various attempts at extensions – to finite-time nonstationary problems, and to vector-valued processes in particular. When observations are only available over a finite time, say $(0, t)$ rather than $(-\infty, t)$, the W-H equation is replaced by one of 'W-H type',

$$h(t, s) + \int_0^t h(t, \tau) \phi(\tau - s) d\tau = \phi(t - s), \quad 0 \leq s \leq t. \quad (9)$$

No general methods were or are known for its solution, and a vast literature developed on various special cases,

tricks, etc; so much so that a 1958 editorial by P. Elias urged no more work on 'Two Famous Papers'. One generic title was 'The Optimum Linear Mean Square Filter for Separating Sinusoidally Modulated Triangular Signals from Randomly Sampled Stationary Gaussian Noise, with Applications to a Problem in Radar'. (The other: 'Information theory, Photosynthesis and Religion'.)

The apparent mess was cleaned up by the use, by R. E. Kalman in 1960, of the *state-space description* of process with rational spectral densities. Such descriptions are actually of much older vintage, see, e.g. a paper by and were used by Wang and Uhlenbeck (1930) on Markov models for noise processes. Doob wrote two long papers on them in 1944 and 1949 but, alas, did not mention them in his very influential 1953 book! Had he done so many developments might have occurred much earlier.

The so-called Kalman (or sometimes Kalman-Bucy) filter has been widely discussed in a host of a papers and textbooks, e.g., Anderson and Moore (1979), Kailath (1981). It gains its power, as just noted, from the introduction of state-space models, which turns out to be equivalent to modelling stochastic processes as linear combinations of the components of a vector-valued Markov process. Briefly, we model a scalar process $s(\cdot)$ with an n -th order rational spectral density as

$$\begin{cases} s(t) = Hx(t) \\ \dot{x}(t) = Fx(t) + u(t) \end{cases} \quad t \geq t_0 \quad (10)$$

where $H \in \mathcal{C}^{1 \times n}$, $F \in \mathcal{C}^{n \times n}$ are known matrices, $u(\cdot)$ is an $n \times 1$ vector-valued zero-mean white noise process, with

$$\langle u(t), u(s) \rangle \triangleq Eu(t)u(s)^* = Q\delta(t-s),$$

and the initial *state*, $x(t_0)$, is such that

$$\begin{aligned} \langle x(t_0), 1 \rangle &= Ex(t_0) = 0, \quad \langle x(t_0), x(t_0) \rangle = \Pi_0, \\ \langle x(t_0), u(t) \rangle &= 0. \end{aligned}$$

The matrices $Q \in \mathcal{C}^{n \times n}$ and $\Pi_0 \in \mathcal{C}^{n \times n}$ are also assumed to be known.

We use the inner product notation to follow Kolmogorov in assuming that (zero-mean) random variables defining a (second-order) stochastic process live in a Hilbert space (or Hilbert module, when the random variables are vector-valued). Of course we are stretching this formulation when we deal with white noise processes, but rigor can be restarted by regarding a white noise process as the formal derivative of a process with orthogonal increments.

Though the linear system relating the stochastic input process $u(\cdot)$ to the output stochastic process is time-invariant, the process $s(\cdot)$ will in general be nonstationary, because the 'transients' arising from the fact that the input is switched on at time t_0 and does not begin in the remote past. In fact, it is not hard to see that

$$\Pi(t) = \langle x(t), x(t) \rangle$$

will obey

$$\Pi(t) = F\Pi(t) + \Pi(t)F^* + Q, \quad \Pi(t_0) = \Pi_0. \quad (13)$$

However, when F is 'stable', i.e., all its eigenvalues have strictly negative real parts, then it turns out that the process $s(\cdot)$ will be stationary if the initial state variance is chosen as

$$\Pi(t_0) = \Pi,$$

where Π is the unique nonnegative definite solution of the (Lyapunov) equation

$$0 = F\Pi + \Pi F^* + Q. \quad (14)$$

Now since we have introduced matrix notation, we can as well take the step of regarding $s(\cdot)$ as a $p \times 1$ vector-valued process, so that $H \in \mathcal{C}^{p \times n}$. The filtering problem is now a 'multichannel' problem of attempting to find

$\hat{s}(t)$ = the linear least-squares estimate of $s(t)$ given $\{y(\tau), t_0 \leq \tau < t\}$,

where

$$y(t) = s(t) + v(t), \quad t \geq 0 \quad (15)$$

and $v(\cdot)$ is a white noise process such that

$$\left\langle \begin{bmatrix} u(t) \\ v(t) \end{bmatrix}, \begin{bmatrix} u(s) \\ v(s) \end{bmatrix} \right\rangle = \begin{bmatrix} Q & S \\ S^* & R \end{bmatrix} \delta(t-s), \quad (16)$$

where $S \in \mathcal{C}^{n \times p}$ and $R \in \mathcal{C}^{p \times p}$ are also assumed to be known *a priori*. The presence of the additive white noise is essential to get useful results, and so it is assumed that R is strictly positive definite, $R > 0$.

It is widely believed that the reason for the greater scope of the Kalman theory (applying to vector-valued but finite-dimensional) stationary processes also to (finite-dimensional) nonstationary process is in fact that it starts, as above, with a model for the process $s(\cdot)$ rather than with power-spectral or covariance data. However this is not true – the Wiener and Kalman approaches become equivalent if one carries over the state-space characterization of the process to its power-spectra and/or covariance functions.

We shall illustrate this now by using the state-space model to solve a problem not satisfactorily resolved by Wiener and several later researchers – finding an effective way of computing the canonical factorization of a rational power spectral density function matrix. We start by noting that since the transfer function from the input white noise processes $\{u(\cdot), v(\cdot)\}$ to the output process $y(\cdot)$ is

$$[H(i\omega I - F)^{-1} \quad I], \quad (17)$$

the power-spectral density function of $y(\cdot)$ can be computed as

$$\Phi_{yy}(\omega) = [H(i\omega I - F)^{-1} \quad I] \begin{bmatrix} Q & S \\ S^* & R \end{bmatrix} \begin{bmatrix} (-i\omega I - F^*)^{-1} H^* \\ I \end{bmatrix}. \quad (18)$$

An alternative expression can be found by taking the Fourier transform of the covariance function:

$$\phi_y(\tau) = \langle y(t), y(t-\tau) \rangle = \frac{R\delta(t-\tau) + He^{F\tau}N1(\tau) + N^*e^{F^*\tau}H^*1(\tau), \quad (19)$$

where

$$N = \Pi H^* + S \quad (20)$$

and $1(\cdot)$ is the (Heaviside) step function

$$1(t) = \begin{cases} 1 & t > 0 \\ 1/2 & t = 0 \\ 0 & t < 0 \end{cases}$$

The Fourier transform of $\phi_y(\cdot)$ is

$$\Phi_{yy}(\omega) = [H(i\omega I - F)^{-1} \quad I] \begin{bmatrix} 0 & N \\ N^* & R \end{bmatrix} \begin{bmatrix} (-i\omega I - F^*)^{-1} H^* \\ I \end{bmatrix}. \quad (21)$$

Comparing (18) and (21) shows that different 'central' matrices could be used to specify $\Phi_{yy}(\omega)$, some nonnegative definite as in (18), while, the one in (21) is indefinite. It is natural to ask how we can characterize the nonuniqueness of the central matrix? The answer is that we can use *any* central matrix of the form

$$M = \begin{bmatrix} Q + FZ + ZF^* & S + ZH^* \\ S^* + HZ & R \end{bmatrix}, \quad Z = Z^* \quad (22)$$

i.e., where Z is any Hermitian matrix. The choices $Z = 0$ and $Z = \Pi$ give the previous expressions (18) and (21). The fact that any such M yields $\Phi_{yy}(\omega)$ can be verified by a direct (but tedious) calculation. However, a nicer and more useful derivation can be obtained by allowing the random variables to live in an indefinite (Krein space), rather than in Hilbert space.

In a Krein space we can have elements such that $\langle u, u \rangle$ is indefinite or even zero. For example, we could have

$$\left\langle \begin{bmatrix} u^0(t) \\ v^0(t) \end{bmatrix}, \begin{bmatrix} u^0(s) \\ v^0(s) \end{bmatrix} \right\rangle \triangleq \begin{bmatrix} Q^0 & S^0 \\ S^{0*} & R^0 \end{bmatrix} \delta(t-s) = \begin{bmatrix} 0 & N \\ N^* & R \end{bmatrix} \delta(t-s). \quad (23)$$

In view of this, let us add to the original $\{u(\cdot), v(\cdot)\}$, elements $\{u^0(\cdot), v^0(\cdot)\}$ such that (in an obvious notation)

$$\begin{cases} \dot{x}(t) + \dot{x}^0(t) = F(x(t) + x^0(t)) + G(u(t) + u^0(t)) \\ y(t) + y^0(t) = H(x(t) + x^0(t)) + v(t) + v^0(t) \end{cases} \quad (24)$$

where

$$\left\langle \begin{bmatrix} u(t) \\ v(t) \end{bmatrix}, \begin{bmatrix} u^0(t) \\ v^0(t) \end{bmatrix} \right\rangle = 0 \text{ and } \langle y^0(t), y^0(\tau) \rangle = 0. \quad (25)$$

This can be done using earlier formulas (see (5)–(6)) to note that

$$\Phi_{y_0 y_0}(\omega) = [H(i\omega I - F)^{-1} \quad I] \begin{bmatrix} 0 & \Pi^0 H^* + S^0 \\ H \Pi^0 + S^0 & R^0 \end{bmatrix} \begin{bmatrix} (-i\omega I - F^*)^{-1} H^* \\ I \end{bmatrix}, \quad (26)$$

where $\Pi^0 = \Pi^{0*}$ is such that

$$F \Pi^0 + \Pi^0 F^* + Q^0 = 0. \quad (27)$$

From these we conclude that $\Phi_{y_0 y_0}(\omega)$ will be identically zero if we choose

$$S^0 = -\Pi^0 H^*, \quad Q^0 = -F \Pi^0 - \Pi^0 F^* \text{ and } R^0 = 0. \quad (28)$$

Finally setting $Z = -\Pi_0$ gives

$$\begin{aligned} & \left\langle \begin{bmatrix} u(t) + u^0(t) \\ v(t) + v^0(t) \end{bmatrix}, \begin{bmatrix} u(s) + u^0(s) \\ v(s) + v^0(s) \end{bmatrix} \right\rangle \\ &= \begin{bmatrix} Q + Q^0 & S + S^0 \\ S^* + S^0 & R + R^0 \end{bmatrix} \delta(t-s) \\ &= \begin{bmatrix} Q + FZ + ZF^* & S + ZH^* \\ S^* + HZ & R \end{bmatrix} \delta(t-s) \triangleq M \delta(t-s) \end{aligned} \quad (29)$$

exactly as claimed above (see (22)). We see now that the arbitrary matrix Z can be interpreted as the (negative of) the state-variance matrix of a process with zero s -spectrum. However, so far we only have a formal calculation. The significant theorem is the so-called KYP Lemma (see Willems and Trentelman for a recent discussion):

Theorem (KYP Lemma) When $\Phi_y(s) > 0$, $s = j\omega$, then there exists a $Z = Z^*$ such that the central matrix is non-negative definite (i.e., it is the covariance matrix of a collection of genuine random variables).

- We do not need F to be stable; a weaker condition from linear system theory, a subject developed in the engineering literature of the last 30 years, will suffice: the pair $\{F, H\}$ should be detectable, i.e., it should be such that $[H^* s I - F^*]$, $s = \sigma + i\omega$, should be full rank for all $\sigma \geq 0$. Here however we shall, for simplicity, stay with the assumption that F is stable so that we are dealing with a stationary process $y(\cdot)$. There are important corollaries characterizing matrix *positive-real* (Caratheodory) and *bounded-real* (Schur) functions, which are widely encountered in applications. We note also that the Krein space interpretation introduced above can be used to give a simple geometric proof of the lemma; it also leads to several other results, e.g., unifying H_2 and H_∞ control (see, e.g., Hassibi *et al.* (1996)).

However while the KYP Lemma is an important result, which is why we mentioned it here, it is not necessary to use it to obtain a spectral factorization of $\Phi_{yy}(\omega)$. To this end, note that although we cannot make any assertions on the positivity of the central matrix M , defined in (28), the fact that

$$\Phi_{yy}(\omega) = [H(i\omega I - F)^{-1} \quad I] M \begin{bmatrix} (-i\omega I - F^*)^{-1} H^* \\ I \end{bmatrix} > 0 \quad (30)$$

shows that M has at least p positive eigenvalues. [Note that, for each ω , the above expression is the product of a $p \times (n+p)$, an $(n+p) \times (n+p)$ and an $(n+p) \times p$ matrix, which is positive definite (i.e., has p positive eigenvalues). Therefore the central $(n+p) \times (n+p)$ matrix, M , must have at least p positive eigenvalues.]

Now that we have shown that the matrix M has at least p positive eigenvalues for all choices of Z , it is interesting to ask whether Z can be chosen so that M has only p positive eigenvalues and no negative eigenvalues, i.e., if Z can be chosen so that M has minimal rank p . To see that this is indeed possible recall the easily verified factorization (recall that we have assumed the invertibility of R).

$$M = \begin{bmatrix} Q + FZ + ZF^* & S + ZH^* \\ S^* + HZ & R \end{bmatrix} = \begin{bmatrix} I & K \\ 0 & I \end{bmatrix} \begin{bmatrix} \Delta & 0 \\ 0 & R \end{bmatrix} \begin{bmatrix} I & 0 \\ K^* & I \end{bmatrix}, \quad (31)$$

where

$$\Delta(Z) \triangleq Q + FZ + ZF^* - (S + ZH^*)(R(S + ZH^*))^* \quad (32)$$

$$K \triangleq (S + ZH^*)R^{-1}. \quad (33)$$

Therefore $\Phi_{yy}(\omega)$ in (30) can be written as

$$\Phi_{yy}(\omega) = H(i\omega - F)^{-1} \Delta(Z) (-i\omega - F^*)^{-1} H^* + [I + H(i\omega - F)^{-1} K] R [I + H(i\omega - F)^{-1} K]^* \quad (34)$$

The second term on the RHS is $p \times p$ and non-negative definite, so we can immediately obtain a factorization by choosing Z so that it satisfies

$$0 = \Delta(Z) = FZ + ZF^* - (S + ZH^*)R^{-1}(S + ZH^*)^* \quad (35)$$

The only issue is whether the resulting spectral factor has a well-defined inverse, viz., one that when extended into the complex plane is analytic in the right half plan (cf. (2)). There is an interesting result here. The nonlinear algebraic equation (35), which for reasons explained below is called an Algebraic Riccati Equation (or ARE), has many solutions. However it can be shown that when F is stable (or even just when $\{F, H\}$ is detectable), there is one and only one non-negative solution, say P ; moreover, this solution is such that the spectral factor

$$\Psi(s) \triangleq [H(sI - F)^{-1} K + I] R^{1/2}, \quad K = S + PH^* R^{-1} \quad (36)$$

and its inverse

$$\begin{aligned} \Psi^{-1}(s) &\triangleq [H(sI - F)^{-1} K R^{1/2} + R^{1/2}]^{-1} \\ &= R^{-1/2} [I - H(sI - F + KH)^{-1} K], \end{aligned} \quad (37)$$

are both analytic in the right half plane. There are several computationally effective methods of finding the desired nonnegative definite solution of the ARE – a good source is the reprint volume edited by Bittanti *et al.* (1995). So the introduction of the ARE, first done in the Kalman theory, overcame what had been regarded as one of the stumbling blocks to the Wiener theory. A minor quibble is that the factorization is expressed in terms of the parameters $\{F, G, H, Q, R, S\}$ of a particular model for the process rather than in terms of the spectral data. Now for the state-space model, the covariance and the spectral density are fixed by (cf. (19)–(21)) by $\{H, F, N\}$. To use this data, all we need to do is to choose the central matrix M not as in (31), but as (cf. (19)–(22)).

$$\begin{bmatrix} 0 + FZ + ZF^* & N + ZH^* \\ N^* + HZ & R \end{bmatrix} \quad (38)$$

Then proceeding as before, the rank of this matrix can be dropped by choosing Z so that it satisfies

$$0 = FZ + ZF^* - (N + ZH^*)R^{-1}(N + ZH^*)^* \quad (39)$$

which will lead to a factorization of the form

$$\Phi_{yy}(s) = [I + H(sI - F)^{-1}(N + ZH^*)]R^{-1}[\dots]^* \quad (40)$$

The particular choice that will give a factor with an inverse that is analytic in the right-hand plane can be shown to be the unique negative semidefinite, say $-\Sigma$, solution of the ARE (39). The corresponding factor is therefore

$$\Psi(s) = [I + H(sI - F)^{-1}K]R^{1/2}, \quad (41)$$

where we define

$$K \triangleq N - \Sigma H^*, \quad (42)$$

and

$$\Sigma \geq 0, 0 = F\Sigma + \Sigma F^* + (N - \Sigma H^*)R^{-1}(N - \Sigma H^*)^* \quad (43)$$

The reader may have wondered that we used the *same* symbols $\Psi(s)$ and K as in the earlier formula (36) – the reason is that the canonical factorization is unique! [This implies the interesting identity $\Pi = P + \Sigma$, which we shall not explore here.]

To close the story of Wiener filtering, let us note that with the canonical factor in hand, we can really write down the optimal filter by using (8) and (37)

$$\mathcal{K}(s) = I - R^{1/2}\Psi^{-1}(s) = H(sI - F + KH)^{-1}K \quad (44)$$

where K can be found either from the model parameters, as in (36), or from the covariance/spectral parameters, as in (42). This is a reasonably explicit formula for the optimal filter, but another advantage of the state-space formulation is that we can readily write down a state-space model for the filter:

$$\dot{\hat{x}}(t) = (F - KH)\hat{x}(t) + Ky(t), \quad \hat{x}(t_0) = 0 \quad (45)$$

$$\hat{s}(t) = H\hat{x}(t) \quad (46)$$

as can be verified by checking that the transfer function from $y(\cdot)$ to $\hat{s}(\cdot)$ is exactly as in (44). We have used the notation $\hat{x}(\cdot)$ for the state-variable in (45), because in fact we have a bonus: $\hat{x}(\cdot)$ is the linear least-squares estimate of the state $x(\cdot)$ itself (under the assumption that $\{F, H\}$ is observable, i.e., $[sI - F^*H^*]$ has full rank for all $s \in C$).

We close with some remarks that, *inter alia*, will fulfill our promise to explain the name ARE. The first remark is obtained by going back to our state-space model, (10) *seq.* Observe that stationarity arose from a particular choice of initial condition, $\Pi(t_0) = \Pi$ defined as the unique matrix such that

$$\Pi \geq 0, 0 = F\Pi + \Pi F^* + Q$$

For any other choice of $\Pi(t_0)$, or if F is unstable (so that (14) will not have a solution $\Pi \geq 0$), the process $s(\cdot)$ will be nonstationary, with covariance function

$$Es(t + \tau)s^*(t) = He^{F\tau}N(\tau), \quad \tau \geq 0$$

where

$$N(t) = \Pi(t)H^*, \quad \dot{\Pi}(t) = F\Pi(t) + \Pi(t)F^* + Q,$$

It turns out that the previous discussions can all be extended by now working in terms of covariance functions rather than power-spectral-density functions. The key change is that instead of the algebraic (Riccati) equation

$$P \geq 0, 0 = Q + FP + PF^*KRK^*, \quad K \equiv (S + PH^*)R^{-1}$$

we shall have the matrix Riccati differential equation,

$$\dot{P}(t) = Q + FP(t) + P(t)F^* - K(t)RH^*(t), \quad P(t_0) = \Pi(t_0)$$

$$K(t) \triangleq (S + P(t)H^*)R^{-1}$$

When the state is one dimensional, the resulting quadratically nonlinear equation is the one apparently first studied by Jacopo Francesco, Count Riccati, and later introduced by Legendre and others into the calculus of variations. It was introduced into control theory by R. E. Bellman (1957) and the matrix version by especially R. E. Kalman (1960).

Explicit analytic solution of the Riccati equation is impossible in the matrix case. But fortunately, this is a (nonlinear) initial value problem, so it can be solved via a discretization scheme, e.g., in the naive way,

$$P(t + \delta) = P(t) + \delta[Q + FP(t) + P(t)F^* - K(t)RK^*(t) + 0(\delta)], \quad t = 0, 1, 2, \dots$$

Now, an important observation is that once the need for some computer-based iterative algorithm is realized, one might further guess that there is no particular need to restrict oneself to time-invariant systems: one can just as easily consider time-variant models,

$$\dot{x}(t) = F(t)x(t) + v(t), \quad t \geq t_0$$

$$y(t) = H(t)x(t) + v(t)$$

with

$$\left\langle \begin{bmatrix} u(t) \\ v(t) \end{bmatrix}, \begin{bmatrix} u(s) \\ v(s) \end{bmatrix} \right\rangle = \begin{bmatrix} Q(t) & S(t) \\ S^*(t) & R(t) \end{bmatrix} \delta(t-s).$$

Now the (Riccati) iteration is still as before,

$$P(t + \delta) = P(t) + \delta[Q(t) + F(t)P(t) + \dots] + 0(\delta), \quad t = 0, 1, 2, \dots$$

except that we need to store/know the values of the functions $\{F(\cdot), Q(\cdot), \dots\}$.

We have thus arrived at the Kalman-(Bucy) filtering algorithm. There is a vast literature on it, with several significant results and issues. Here, we go on to a different kind of extension of Wiener's results, involving finite-time discrete time series. That discussion will lead us to a concept called *displacement structure*, which actually had its roots in studies of the Riccati equation.

7. Beyond state-space models/displacement structure

In one of several different variations of Wiener's problem, his colleague N. Levinson in 1947 studied a finite-time discrete prediction problem, where the Wiener-Hopf equation was replaced by a set of linear equations with a Toeplitz coefficient matrix. He proposed a fast recursive solution, now known as the *Levinson algorithm*, very widely used in geophysical data processing (beginning in the mid-fifties) and in speech processing (beginning in the mid-sixties). Kolmogorov's (1939) formulation of the prediction problem gives an interesting insight into this algorithm, and led to connections with the work of Szegö (1939) and Geronimus (1939) on orthogonal polynomials, of Schur (1917) on H^∞ functions, and then to new results on Toeplitz-like matrices and more generally matrices with displacement structure (see below).

The Kolmogorov Isomorphism: The identity

$$\langle y_k, y_l \rangle = E_{y_k y_l^*} = r_{k-l} = \oint z^k z^{-l} \frac{dF(z)}{2\pi z} = \langle z^k, z^l \rangle_2$$

allows one to form an isometric mapping between the Hilbert space of random variables spanned by $\{y_k\}$, and the Hilbert space of functions on the unit circle spanned by the $\{z^k\}$.

Then the finite-interval prediction problem: find $\{a_{k,j}\}$ to minimize

$$E \|y_k + a_{k,1}y_{k-1} + \dots + a_{k,m}y_{k-m}\|^2$$

is equivalent to the polynomial approximation problem: find $\{a_{k,j}\}$ to minimize

$$\oint |z^k + a_{k,1}z^{k-1} + \dots + a_{k,m}z^{k-m}|^2 \frac{dF(z)}{2\pi z}.$$

It turns out that around 1920 Szegö had shown that the minimizing polynomials

$$a_m(z) = z^m + a_{m,1}z^{m-1} + \dots + a_{m,m}$$

had the nice property that they were orthogonal to each other w.r.t. the measure $F(z)$. Szegö and others went on to make many studies of these orthogonal polynomials. Among other results, in 1939, Szegö and Geronimus independently discovered that these polynomials obeyed a two-term (rather than the usual 3-term) recursion:

$$a_{m+1}(z) = a_m(z) - k_{m+1}z a_m^{\#}(z), \quad a_m^{\#}(z) = \text{the reciprocal polynomial}$$

where $k_{m+1} = -a_{m+1,m+1}$, the constant term in $a_{m+1}(z)$. This is in fact almost the same as the recursion discovered by Levinson in 1947, except that to obtain a true recursion one needs to be able to compute k_{m+1} in terms of $\{F(z), a_m(z)\}$. This could have been done by Szegö or Geronimus, had they been interested in actual computation; however they were more interested in the asymptotic properties of the polynomials (in fact, a famous Szegö formula is just the formula discovered by Kolmogorov and Wiener for the irreducible error in prediction). A survey of the connections between orthogonal polynomial theory and linear estimation, and their fascinating continuous-time analogs, can be found in Kailath *et al.* (1978).

Later it was discovered that a more farreaching connection could be made with some of the work of I. Schur, who was well ahead of his time with his interest in computation. In 1917, he wrote a remarkable paper giving a computationally efficient solution to the Caratheodory moment problem that, in effect, also gave a fast algorithm for factorizing Toeplitz matrices; Levinson's algorithm factorizes the *inverse* of a Toeplitz matrix. It turns out that Schur's algorithm offers an alternative to the Levinson algorithm: it is somewhat slower for serial computation, but can be much faster for (software or hardware) parallel implementation!

There are many aspects to these algorithms arising from pursuing the prediction problem. One of the most fascinating is the concept of *displacement structure*. One way of motivating it is by asking questions such as the following:

If there are fast algorithms for factorizing Toeplitz matrices, what about factoring non-Toeplitz matrices that are known to have Toeplitz inverses? Similarly, should it be much harder to factor the non-Toeplitz matrix $T_1 T_2$ or $T_1 T_2^{-1} T_3$ than T_1 (or T_2 or T_3) alone?

The answer is that these problems in fact have the same order of complexity as purely Toeplitz problems do. The reason is that what allows fast algorithms for Toeplitz matrices is not their Toeplitzness, which is lost under inversion and under multiplication, but something called *displacement structure*: R has displacement

structure if $R = FRA$, or more generally $\Omega R \Delta = FRA$ has low rank for appropriate (low complexity) matrices $\{\Omega, \Delta, F, A\}$. The interested reader can verify that when $F = A^* = Z$, the lower shift matrix with ones on the first subdiagonal and zeros elsewhere, a Hermitian Toeplitz matrix and its inverse have displacement rank less than or equal to 2. It is not hard to show that products, inverses and Schur complements essentially inherit the displacement structure. This fact can be exploited to obtain a generalized Schur algorithm for the fast recursive factorization of such matrices. Moreover there is a very useful physical structure – a cascade network or generalized transmission line – that can be associated with the generalized Schur algorithm, a fact that has lots of implications and applications. We may mention, among others, problems in linear algebra, inverse scattering, coding theory, complex interpolation, matrix completion, etc. Surveys of these results can be found in Kailath (1987) and Kailath and Sayed (1995).

To end this account, though we should note that the initial stimulus for the development of the concept of displacement structure came not from linear algebra, but from the Wiener-Hopf equation itself, as it was further studied by the astronomer V. A. Ambartsumian (1943) in the former Soviet Union, and S. Chandrasekhar in the USA. It will take too long to make those connections here, and we refer the interested reader to the reviews Kailath (1991), Sayed and Kailath (1995) for some the history and for some of the later developments, including links back to the work of I. Schur.

8. Nonlinear estimation

In the late 1950s, Wiener gave a series of lectures on the problem of nonlinear least-mean squares estimation, which were transcribed into a monograph (Wiener (1958)). Wiener proposed to use a so-called 'Volterra series' characterization of nonlinear systems as a sum of linear + quadratic + ... systems. However this approach had several limitations, especially of computational complexity and the difficulty of approximation (how many or which terms should we keep for a particular nonlinear system?).

The success of the state-space models for the linear problems led to a significant effort to try to obtain similar results for the nonlinear case. Thus suppose we have a nonlinear system, in state-space form,

$$\begin{cases} \dot{x}(t) = f(x(t), t, u(t)), t \geq 0 \\ y(t) = h(x(t), t) + v(t) = s(t) + v(t), \text{ say.} \end{cases}$$

The minimum mean-square estimator of $x(t)$ given $\{y(\tau), \tau < t\}$ is no longer linear, and its computation requires full statistical knowledge of the non-Gaussian processes $x(\cdot)$, $z(\cdot)$ and $y(\cdot)$:

$$\hat{s}(t) = E[s(t) | \mathcal{F}\{y(\tau), \tau < t\}].$$

When $\{x(\cdot), s(\cdot), y(\cdot)\}$ are jointly Gaussian, one has the Kalman filter recursions. But in general, all has an ascending chain of coupled nonlinear equations for which no really satisfactory practical algorithms, or approximations, have been found. Therefore the nonlinear problem is effectively still open.

However there have been several interesting theoretical results. One set arises from the introduction of ideas from martingale theory (with some of the results now being pursued in finance theory and on Wall Street). Martingale theory first enters through the fact that the white Gaussian measurement noise, $v(\cdot)$, of the engineers is the formal derivative of the special process introduced by Wiener in his study of Brownian motion:

$$\int_0^t v(\tau) d\tau = W(t), \text{ the Wiener (-Lévy) process.}$$

The martingale properties of $W(\cdot)$ lead to a striking generalization of the innovations process first introduced in the linear theory. Let us recall from § 5 that with (scalar) observations containing white noise,

$$y(t) = s(t) + v(t), \langle v(t), v(\tau) \rangle = \delta(t - \tau)$$

the optimum linear filter for finding $\hat{s}(\cdot)$ has transfer function (note that now $R \equiv 1$ in (8))

$$K(\omega) = 1 - \Psi^{-1}(\omega).$$

This implies in the time domain that

$$\hat{s}(t) = y(t) - e(t)$$

or that the innovations can be expressed as

$$e(t) = y(t) - \hat{s}(t).$$

Now when we deal with nonlinear operations on white noise, the formal manipulations become harder to justify: linear operations on white noise give smoother processes, but what is the square of white noise? Therefore one now works with integrated processes,

$$Y(t) \triangleq \int_0^t y(\tau) d\tau = \int_0^t s(\tau) d\tau + \int_0^t v(\tau) d\tau = \int_0^t s(\tau) d\tau + W(t)$$

and uses the Ito theory of stochastic integrals, especially as developed by the Japanese and French schools (see e.g., Meyer (1975)).

In this language, one can show (see Kailath (1971), Meyer (1973)) the following: Let

$$Y(t) = \int_0^t s(\tau) d\tau + W(t),$$

with

$$\int_0^T E|s(t)| dt < \infty, E[W(t) - W(\tau)z(\tau)] = 0, \quad t > \tau.$$

Then, the process $E(\cdot)$ defined as

$$E(t) = Y(t) - \int_0^t \hat{s}(\tau) d\tau$$

where

$$\hat{s}(t) \triangleq E[s(t)F\{y(\tau), \tau \leq t\}]$$

is also a Wiener process w.r.t. the (nested) family of sigma fields $\{F\{Y(\tau), \tau \leq t\}\}$. The main idea of the proof is to show first that $E(\cdot)$ is a martingale with respect to these sigma fields, and then to show that $E(\cdot)$ and $W(\cdot)$ have the same 'quadratic variation' (again a concept introduced by Wiener). Then a theorem of Levy's gives the result. This is a nice result, since the process $y(\cdot)$ might be much more complicated than $E(\cdot)$; it shows the power of the assumption of additive white noise. Now in the linear case, results from the theory of integral equations enable us to show that (Kailath (1968, 1972))

$$F\{E(\tau), \tau \leq t\} = F\{Y(\tau), \tau \leq t\}, 0 \leq t \leq T$$

so that the process $\{Y(\cdot)\}$ and $\{E(\cdot)\}$ are replaceable each by the other, without any loss of information. As mentioned earlier, this was the idea behind the innovations approach to the Wiener filter (Bode-Shannon (1950), Zadeh-Ragazzini (1950)); in the nonstationary finite-time case, the above result allows for a similar approach to the Kalman filter and several related problems (Kailath (1970), Davis (1977)).

Therefore an important question is under what conditions this equality of sigma fields holds in the general case. The problem turned out to be quite difficult (Benes (1976)) and only after attempts by several researchers, did Allinger and Mitter (1981) succeed in proving the equality for the case where $s(\cdot)$ and $W(\cdot)$ are independent of each other and $\int_0^T E[s(t)]^2 dt < \infty$.

However even without the equivalence, the process $E(\cdot)$ leads to several nice results. One is that even though the sigma fields generated by $E(\cdot)$ and $Y(\cdot)$ may not be equivalent, Fujisaki *et al.* (1972) showed that any function measurable w.r.t. the Y sigma fields can be written as a stochastic integral w.r.t. the Wiener process $R(\cdot)$. This then allows for a simpler description of the nonlinear filtering equations: however as mentioned before, they are not useful for actual computation.

Another application that exploits only the fact that $E(\cdot)$ is a Wiener process is a generalized Cameron-Martin formula for the Radon-Nikodym derivative of the measures P_y and P_w induced by the processes $Y(\cdot)$ and $W(\cdot)$:

$$\frac{dP_y}{dP_w} = \exp \int_0^T \hat{s}(t) dY(t) - \frac{1}{2} \int_0^T |\hat{s}(t)|^2 dt.$$

This expression has useful implications for the problem of detecting the presence or absence of a random signal $s(\cdot)$ in the presence of noise. When the signal $s(\cdot)$ is deterministic (and therefore known *a priori*) $\hat{s}(\cdot) \equiv s(\cdot)$, this is a result of Cameron and Martin (1944). It is an interesting and useful fact that for random $s(\cdot)$ the deterministic formula still applies with the

unavailable random signal $s(\cdot)$ being replaced by the observable least-squares estimate $\hat{s}(\cdot)$. This allows a lot of the insights and results of estimation theory to be carried over to signal detection theory (Kailath (1969), Davis and Andreidakis (1977)). We may remark that the Cameron-Martin formula arose as a theory of 'linear changes of variables' in Wiener space (the space of sample functions of a Wiener process). The generalized Cameron-Martin formula follows from a nonlinear version of this theory introduced in a seminal paper of Girsanov (1960), which has since been much studied and extended.

9. Concluding remarks

This has been an account of some of the ways in which Norbert Wiener's work and ideas have influenced several engineering developments. The key ideas were his emphasis of the statistical nature of the communication process and his introductions of the use of optimization criteria into system design. I should hasten to add that many other notable researchers (Shannon, Rice, Tukey, Bellman, Pontryagin, Kalman, to name just a few) had major roles in the post-1942 story. Finally, Wiener's own specific mathematical contributions to mathematical engineering are too numerous to cover in a simple article. Here I have described, in a very sketchy way and with some focus on things I know best, some of the wide range of ideas and techniques stimulated by Wiener's work on prediction and filtering.

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Heat shock proteins – Role in thermotolerance of crop plants

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Thermotolerance is required in crop plants in order to maintain productivity under heat stress. At the cellular level, thermotolerance is linked with the induction of heat shock proteins (HSPs), a response conserved from prokaryotes to eukaryotes. HSPs belong to six families and each family has several members, of which only a few may be involved in acquired thermotolerance. Molecular approaches may help to assign specific role to HSPs involved in thermotolerance. Thermotolerant genotypes show adaptations at various levels of organization besides showing qualitative and quantitative differences in HSPs as compared to the thermosensitive genotypes. In future, HSPs and enzymes with broader thermal kinetic windows may be the desired selection criteria at molecular level for breeding thermotolerant crop plants.

AGRICULTURE production must continue to meet the demands of the growing population. In the past decades agricultural production increased due to higher yields and by bringing more land under agricultural production. The scarcity of productive agricultural land may force us to grow agricultural crops in harsher environments. Temperature is an important environmental factor affecting crop productivity. Crops have high productivity when grown in temperatures optimum for various growth and metabolic processes. Temperatures higher than the optimum decrease both the rate and duration of metabolic processes and thus decrease the yield.

Being poikilothermic, plants have to keep their temperatures below ambient through transpiration. In water-limited environments, transpiration is reduced, thus plants experience both water and high temperature stress. Howard¹ remarked that, 'Wheat production in India is a gamble in temperature'. Even today, the wheat season in North India is limited by temperature at both ends of the crop growth. It has been observed that for every one degree rise in mean temperature over the range of 12.2–27.5°C the wheat yield is reduced by 4%. Therefore, to sustain the agricultural production it is necessary to breed varieties which are tolerant to high temperature stress. Further, the current estimates of global warming predict an increase of 0.5°C annual

mean temperature by 1995–2005, 1.5°C by 2050 and 3°C by 2050–2100 AD. These changes in mean values of temperature may be accompanied by high frequencies of extreme levels of heat and moisture.

Thus, it is necessary to understand the physiological and molecular basis of high temperature stress tolerance. In this review, we focus on role of heat shock proteins in high temperature stress tolerance and productivity of crop plants.

Heat shock proteins

Heat stress (5–10°C above the normal growing temperature of organism) induces expression of specific gene families called heat shock genes (*hsps*), which lead to the synthesis of a new set of proteins called heat shock proteins (HSPs). After Tissieres *et al.*² demonstrated for the first time that heat-induced chromosomal puffing of *Drosophila melanogaster* was accompanied by the high level expression of an unique set of proteins called heat shock proteins, HSPs have been found in every organism in which it has been sought from unicellular prokaryotes to highly evolved complex multicellular organisms including *Homo sapiens*. In fact, HSP induction upon heat shock is a highly conserved universal genetic response among organisms from Antarctic algae to archaeobacteria^{3–5}.

The heat shock response of all organisms shares the following common features:

1. Immediately following the heat shock, a new set of unique HSPs are synthesized from newly-transcribed mRNAs.
2. Heat treatments, which induce HSP synthesis, also lead to acquired thermotolerance, i.e. the ability of an organism to withstand a normally lethal temperature if it is first given a heat shock at non-lethal temperature.

Heat shock proteins are classified into two broad categories based on their expression. The heat shock-inducible proteins are called HSPs while the HSP homologues, which are expressed in the cell during normal cell growth and differentiation, are called Heat Shock Cognates (HSCs). Based on their molecular

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Table 1. HSP families

Family	Expression	Location	Functions
HSP110			
HSP104-yeast	Heat inducible	Cytosol	Thermotolerance,
HSP101-soybean		Nucleus	EtOH tolerance
- <i>Arabidopsis</i>			
-rice	ABA		
Casinoytic protease			
(CLP)-pea, tomato			
- <i>Arabidopsis</i>			
ERD 1- <i>Arabidopsis</i>	Dehydration		
HSP90			
80-90 kDa	Constitutive	Cytosol	
HSP81,82- <i>Arabidopsis</i>	Heat inducible	ER	Chaperone
HSP90 - <i>Brassica</i>		Nucleus	
HSP82-yeast			Thermotolerance essential for viability
HSP70			
Dnak (63-79kDa)	Constitutive	Nucleus	Thermotolerance, negatively regulated hsp expression
HSP70-soybean, maize	Heat inducible	Cytosol	molecular chaperone
HSC70-tomato, pea		Mitochondria, Chloroplast	
SSA, SSB, KAR2-yeast			
HSP60			
53-62 kDa	Constitutive	Nucleus	Molecular chaperone
GroEL homolog	Heat inducible	Chloroplast	
HSP60-maize	Developmental		
HSP20			
10-30 kDa	Heat inducible	Nucleus	Protection of organelle macromolecules
HSP20-soybean	Constitutive	Cytosol	
wheat, carrot	Developmental	Mitochondria	
petunia		Chloroplast	
<i>Vigna sinensis</i>	Heat inducible	Chloroplast	
Ubiquitin			
8.5 kDa-maize	Constitutive	Cytosol	Proteolysis
-wheat	Heat inducible		
- <i>Arabidopsis</i>			

weight, HSPs (and HSCs) are grouped into six families (Table 1). The high molecular weight HSPs predominate in prokaryotes, yeast and animals. In higher plants the low molecular weight HSPs predominate and may play crucial role in heat stress tolerance⁶. All the HSPs are coded by nuclear genome except a HSP20 family member of *Vigna sinensis*, which is coded by chloroplast genome.⁷

Mechanism of protection

The role of HSPs during normal cell growth and development in the proper folding of polypeptides, formation

of multimeric enzyme complexes and transport of proteins to the proper site/organelle has been well studied⁵. Historically, HSPs are believed to prevent the accumulation of aberrant proteins generated as a result of exposure to high temperature or other forms of stresses. Evidences available so far indicate that HSPs protect proteins from denaturation, salvage the denatured proteins and target the aberrant proteins for proteolysis. In soybean, both high and low molecular weight HSPs protected soluble proteins from heat denaturation⁸ and the degree of protection showed dose-dependent character and did not require any additional energy⁹. Repair of heat damaged/denatured proteins is essential for both

survival and recovery from heat stress. HSP104 of yeast, which is essential for survival under heat stress, functions by reactivation of heat-damaged proteins. Thus HSP104 repaired the denatured luciferase to the control level *in vivo* within two hours¹⁰. This function of HSP has been confirmed *in vitro*, but needs to be confirmed *in vivo* in higher plants. Similarly, HSP60 from etiolated *Avena sativa* seedlings stimulated the refolding of chemically-denatured phytochrome to a photoactive form within an hour. The reactivation required additional energy as ATP¹¹. Proteolysis of denatured proteins is another strategy, used by cells to prevent accumulation of denatured proteins. Ubiquitins which target the damaged proteins for proteolysis are coded by multigene family¹² and show heat induction¹³ in higher plants. (In wheat (*T. aestivum* L. cv. Len), heat stress elevated the levels of ubiquitin-protein conjugates, induced HSP synthesis and elevated degradation of soluble proteins.) These results indicate that ubiquitin targets the denatured proteins for proteolysis under heat stress¹³. The protection of damaged proteins is less energy dependent while proteolysis is more energy dependent.

Regulation of HSP expression

The remarkable conservation of heat-stress response from prokaryotes to eukaryotes includes not only the structure and function of the HSPs but also the control of their stress-dependent expression. The environmental stimuli that induce *hsps* other than heat stress are toxic metals, inhibitors of energy metabolism, amino acid analogues, etc. Although HSP inducers are bewildering in their variety¹⁴⁻¹⁷, many of them have in common the capacity to cause protein denaturation. Hightower¹⁸ suggested that the accumulation of denatured or abnormally-folded proteins in cells initiated a stress response and the stress proteins might somehow facilitate the identification and removal of denatured proteins. This proposal was confirmed when Ananthan *et al.*¹⁹ showed that injecting denatured proteins into living cells was sufficient to induce *hsp* genes in eukaryotes.

In eukaryotes, the induction of transcription of *hsps* is mediated by pre-existing transcription factors, the heat shock factors (HSF). HSFs are transacting factors, which upon activation bind to heat shock promoter elements (HSEs) at the 5' upstream end of *hsps*, and induce the *hsp* expression^{5,20}. The binding motif of HSE is composed of 5 bp (nGAAn) blocks in alternating orientation and at least three units are required for stable binding²¹. In higher organisms, binding of HSF to HSE is heat stress inducible^{22,23} and requires conversion from a latent monomer to an active trimer²⁰. HSF genes have been isolated from the yeast (*Saccharomyces cerevisiae* K. lactis), higher plants (tomato and *Arabidopsis*), fruit

fly, chicken, mouse and man. HSFs have two highly-conserved regions: an NH₂-terminal DNA binding domain of ~100 amino acids and an adjacent trimerization domain containing 3 hydrophobic heptad repeats, leu zippers. For the higher eukaryotes there is a fourth zipper domain near COOH-terminus that appears to interact directly with the more NH₂-terminal Leu zippers array to prevent trimerization under non-stress condition⁵. HSF is encoded by a single gene in yeast and *Drosophila* and by three genes in tomato²³. Using tomato HSF genes (*hsfs*) as heterologous probes, *Arabidopsis hsf1* has been cloned²⁴. Sequence comparison of *hsf* genes from different species demonstrates a strong conservation but only in their DNA binding and oligomerization domains. Out of three *hsfs* of tomato, *hsf8* is constitutively expressed, while *hsf24* and *hsf30* are induced by heat stress²⁵. The *hsf1* is also constitutively expressed but the level is increased to two to threefold upon heat shock.

HSF activation may also involve cellular factors as intermediary sensors to regulate activity of HSFs under non-stress conditions. HSFs are maintained in monomeric form through transient interaction with HSP70 and/or other HSPs which are constitutive. During heat shock, due to the availability of denatured/misfolded proteins, which are substrate for HSPs, they release HSFs and bind to denatured proteins. These free HSFs can then form trimers to bind HSEs^{5,20}. Thus in cells, a homeostatic mechanism involving the free level of HSPs (=HSP70) provides a thermometer for reacting to temperature changes. HSFs which are expressed during normal temperature and heat stress are different and heat-induced phosphorylation of some HSFs suggests that other kinds of activation of HSFs also occur in cell²⁶. Species-specific expression of *hsps* may be regulated by the specific upstream sequences on the *hsp* genes, as in case of barley *hsp17* (ref. 27).

Role of HSPs in thermotolerance of higher plants

The induction of HSPs is characteristic of an emergency response, i.e. they are extremely rapid and very strong. For example, in *Glycine max* seedlings, *hsp* mRNAs are observed within 5 min of heat shock, and up to 20,000 fold induction of HSPs occurs²⁸. Secondly, the induction temperature reflects stress conditions for the organism (Table 2).

The plant species adapted to temperate environment (soybean, maize, pea and wheat) begin to synthesize HSPs when tissue temperature exceeds 32–33°C (ref. 29). Thus the HSP-inducing temperature of these organisms reflects the thermal characteristic of the environment in which the organisms are growing. Under field conditions, soil water deficit enhanced midday canopy

Table 2. *hsp* induction temperature of organisms

Organism HSP maximum	Growth temperature optimum (°C)	Induction temperature (°C)
I. Archaeobacteria		
Extreme thermophile <i>Pyrodichim occultum</i>	102	108
II. Prokaryote: <i>E. coli</i>	38	42
III. Eukaryotes		
1. Arctic fishes	0	5-10
2. <i>Drosophila</i>	25	33-38
3. Birds	25-35	43-45
4. Mammals		45
5. Snow fungus <i>Fusarium nivale</i>	12	25
6. Antarctic algae <i>Plocamium cartilagineum</i>	0	5
7. Yeast <i>Saccharomyces cervisiae</i>	25-28	37
8. Higher plants		
a) <i>Lolium temulentum</i>	25-30	35
b) <i>Triticum</i> sp.	20-25	32-40
c) <i>Glycine max</i>	28	40
d) <i>Sorghum bicolor</i>	35	43-45
e) Maize	35	43-45
f) <i>Pennisetum glaucum</i>	35	45
g) Tomato	25	35-37
h) Cotton	30	40
i) <i>Arabidopsis thaliana</i>	22	35

temperature of 40°C induced HSPs in cotton³⁰. Even in irrigated wheat, HSPs were expressed in the field condition when the flag leaf temperature reached 32-35°C and HSP expression was correlated with the thermotolerance³¹. Hence HSPs are expressed in these organisms, when their temperature increases above the normal, rather than at a universal temperature threshold. The kinetics of HSP induction and acquired thermotolerance are tightly coupled and highly conserved among the evolutionarily diverse organisms. Moreover, HSPs show high similarity in nucleotide and amino acid sequences among eukaryotes, and in some cases also with prokaryotes. These evolutionary conservations clearly suggest the importance of HSPs in heat stress tolerance of organisms.

Correlation between HSP expression and thermotolerance

Acquired thermotolerance is correlated with HSP synthesis in many organisms, including higher plants^{3,29,32}. Using etiolated soybean seedlings Lin *et al.*³³ have shown that the rate of synthesis of low molecular weight HSPs is correlated with acquired thermotolerance. Soybean seedlings are able to acquire thermotolerance by a pre-treatment of 2 h at 40°C or 10 min at 45°C followed by 2 h at 25°C. Thus, the pre-heat shock temperature which induced HSP synthesis resulted in the tolerance of

seedlings at 45°C, 2 h heat shock. The 40°C pre-heat shock not only induced the HSP synthesis in soybean seedlings, but also resulted in the localization and stable association of HSPs with cell organelle fractions (nuclei, mitochondria and ribosome). Similarly in other crops like wheat³⁴⁻³⁷, sorghum, pearl millet^{38,39}, and maize⁴⁰, the seedling thermotolerance at otherwise lethal temperature is correlated with the kinetics of HSP synthesis. These studies strongly suggest that the accumulation of HSPs is important for protection from thermal killing. If so, the next question arises, whether the quantity of HSPs or the quality of HSPs is important in providing thermotolerance. *Triticum monococcum* L cultivars, M₃ and M₉, which differ in thermotolerance, did not differ in the quality of HSP induced at 37°C. But Northern analysis using HSP cDNAs as probes revealed that the tolerant genotype M₃ was able to accumulate higher steady state mRNA level of 16.9, 26 and 70 kDa HSPs than the heat susceptible M₉ during heat hardening period⁴¹.

In *Triticum aestivum*, the heat tolerant variety Mustang maintained its cell viability up to 80% when pre-heat shocked (at 34°C) wheat leaves were exposed to 50°C for 1 h, while the susceptible genotype Sturdy could maintain only 40% cell viability. Mustang also maintained its capacity to synthesize a small subunit of Rubisco at 34°C, while Sturdy could not. Two-dimensional gel electrophoretic analysis revealed the presence of three unique HSPs (16, 17 and 26 kDa) in

Mustang³⁵. In case of maize also, the drought and heat-tolerant genotype ZPBL1304 synthesized an unique 42 kDa HSP, which was absent in susceptible line ZPL389 (ref. 40). Thus, not only are the HSP synthesis and acquired thermotolerance tightly coupled, but also the intraspecific differences in quantity, quality and the rate of accumulation of HSPs are highly correlated with thermotolerance.

Cellular localization of HSPs: Heat shock dependent

The positive correlation between acquisition of thermotolerance and HSPs appears to depend not only upon synthesis of HSPs but also on their cellular localization^{33,42,43}. In soybean, heat hardening, which induced seedling thermotolerance, also induced synthesis and selective localization of HSPs. Cell fractionation studies revealed that the low molecular weight 15–18 kDa HSPs selectively localized and associated with nuclei, mitochondria and ribosomes, a lesser amount of 68–70 and 90 kDa HSPs localized in these organelles. While some 22–24 kDa HSPs remained soluble in cytosol, they remained organelle associated during a chase at 40°C, but dissociated gradually during a chase at 28°C. If again 10 min heat shock at 45°C was given, the localization occurred within 15 min. Arsenite-induced HSPs did not localize at 28°C, but they became organelle associated during subsequent heat stress³³.

Similarly, pea HSP22 was strongly associated with chloroplast thylakoids when the temperature was raised above 38°C, at high light intensities^{44–46}. Studies conducted in *Chlamydomonas* showed association of LMW HSP with PS II which protected the PS II from photo-inhibition⁴⁷. In higher plants too, the localization and association of HSPs with organelle demonstrated the protective role of HSPs during heat stress. In soybean seedlings, at 38°C, all HSPs were synthesized, but their organelle localization occurred at 42.5°C. The 15–18 kD HSP and 70 kDa HSP were associated with mitochondria at 42.5°C and dissociated during 4 h recovery period at 20°C. This association protected the mitochondrial phosphorylation at non-permissive temperatures⁴⁸.

Cross tolerance and HSPs

HSPs are induced by many other stresses such as ethanol, malonate, arsenite³³, amino acid analogues⁴⁹, abscisic acid^{50–52}, drought stress⁵¹, wounding⁵⁰, γ -rays⁵³ and cold⁵⁴. If thermotolerance is a direct result of HSP synthesis, then the other treatments which induce HSP synthesis should also induce thermotolerance. Arsenite induced HSP synthesis at 28°C in soybean and was able to provide thermotolerance to the seedlings³³. Also, the

arsenite-induced HSPs showed heat-induced localization and association with organelles. In 40-day-old seedlings of sorghum and pearl millet, arsenite (100 μ M) and malonate (25 mM) induced HSPs synthesis comparable to that of 45°C heat hardening induced HSPs, which also gave thermal protection to the protein synthesis and seedling growth at 50°C³⁸. Ethanol (4%, 6%) which was able to induce HSP synthesis and provide cross tolerance in yeast⁵⁵, was unable to induce either HSPs or thermotolerance in sorghum and pearl millet³⁸. Cell free fractions (soluble proteins) isolated from control and ABA treated cells of *Bromus inermis* Leyss showed different temperature tolerance in temperature-induced protein coagulation assay. Addition of 50 μ g (5%) of ABA induced heat stable proteins and decreased the rate of heat-induced coagulation of cell free fractions *in vitro*⁵⁰. Thus, it seems logical to conclude that HSPs must be playing an important role in thermal protection of higher plants.

Genetic complementation

Not only the heat shock response, but also the HSPs themselves and their regulation of expression is highly conserved among evolutionarily diverse organisms^{20,29}. HSP101 of *Arabidopsis* and soybean show 43% identity to *Saccharomyces cerevisiae* HSP104 at the amino acid level. The conservation of structure and mode of expression suggests that the functionality must have also been conserved. To test this possibility, yeast HSP104 mutants, which did not acquire thermotolerance⁵⁶, were transformed with *Arabidopsis thaliana* hsp101 and soybean HSP101 gene. Higher plant HSP101 is undetectable in yeast (transformed with *Arabidopsis thaliana* hsp101/*Glycine max* hsp101) in the absence of heat stress, but accumulated to high levels during exposure to high temperature. Both *Arabidopsis thaliana* HSP101 and *Glycine max* HSP101 are able to complement the thermotolerance defect caused by HSP104 gene mutation^{57,58}. HSP104 in yeast functions in thermotolerance by promoting the reactivation of heat-damaged proteins after high temperature stress¹⁰. Since higher plant HSP101 was able to complement the thermotolerance deficiency of hsp104 mutant yeast strain, it seems plant HSP104 is able to functionally complement the HSP104 of yeast. Therefore, it appears that HSP104 provides thermotolerance to higher plants in a manner which is functionally similar to that of yeast HSP104.

Molecular biology approaches

Molecular biological approaches were used to prove the role of HSPs in thermal stress tolerance in several organisms. HSP mutations in yeast⁵⁶ and *E. coli*⁵⁹ resulted in temperature sensitivity. Complementation of the yeast

hsp104 mutation by either yeast or higher plant HSP101 resulted in restoration of acquired thermotolerance^{57,58}. Over-expression of HSP70 in *D. melanogaster* resulted in faster acquisition of thermotolerance⁶⁰. Similarly selection of thermotolerant cell lines of Chinese hamster fibroblast cell showed high level expression of HSP70 (ref. 61). Competitive inhibition at transcriptional level of *hsp70* gene in Chinese hamster ovary (CHO) cells reduced the heat-induced expression of *hsp* by at least 90%, which resulted in elevated thermosensitivity⁶². Expression of *hsp27* gene from metallothionein-regulated promoter in CHO cells, conferred metal regulated thermotolerance⁶³. Affinity purified monoclonal antibodies to HSP70 when introduced into human fibroblasts by microinjection impaired heat-induced translocation of HSP70 into nucleus after mild heat shock and rendered the cells thermosensitive⁶⁴. *D. melanogaster* and mammalian cells transformed with *hsp70* and *hsp90* antisense genes respectively, accumulated HSP70 at a slower rate and showed reduced thermotolerance^{60,65}.

These studies conclusively prove that some or other kind of HSP is involved in the protection of cells under high temperature stress. These kinds of molecular approaches have been limiting in higher plants because of

1. Existence of *hsp* multigene families showing high homology among the members.
2. Polyploidy nature of several plant species.
3. Lack of knowledge of roles of each HSP family under normal/stress environment.

However, Schöffl⁶⁶ suggested two gene manipulation strategies for HSP analysis in higher plants which includes:

a) Selection of cells and plants with constitutively repressed gene for which antisense mRNA approach appears to be more promising because within the members of a family ~90% homology is present. Hence, it should be possible to repress the expression of all family members by a temperature dependently transcribed antisense mRNA of single gene.

b) Generation of plants that overexpress the desired HSPs, which will be useful to examine the biological effect of protein dosage, protein structure and changed specificity under thermal stress.

Already studies have been initiated to study the regulation of HSP expression in higher plant using 1) GUS gene fused with *hsp* promoter⁶⁷. 2) Soybean *hsp70* fused with *Drosophila hsp70* promoter⁶⁸, which showed regulated expression of HSPs environmentally and developmentally. Efforts have also been made to over-express/to inhibit synthesis by antisense mRNA approach. The tobacco transgenic tobacco plants developed by soybean HSP17.6 fused with cauliflower mosaic virus 35S promoter expressed constitutively to the level comparable

to that of heat induction⁶⁹. However, upon heat shock in the transgenic tobacco plants Gm HSP 17.6 was inhibited which indicated that CaMV35S promoter was not transcriptionally competent under heat stress. In antisense transgenic tobacco (soybean *hsp17.6* fused with cauliflower mosaic virus 35S promoter in the antisense orientation) the level of expression was very low, the reasons being the long distance of the inverted gene from its promoter site and lack of a suitable 3' termination signal of transcription⁶⁹.

Developmental expression of HSPs

All the cells/tissues so far examined are capable of synthesizing HSPs in response to heat shocks, except germinating pollen^{70,71} and pre-torpedo stage of very early embryo development⁷². So, in all other stages of plant development, HSPs are expressed in response to heat stress. The question then asked is, are HSPs developmentally regulated in the absence of heat stress? Studies from eukaryotes including plants clearly indicate that there is, in fact, a tissue and developmental specificity in the expression of HSPs. Expression of HSPs in optimal growth environments occurs in flowers, pods and seeds of pulses⁷³, sepals, filaments and styles of transgenic *P_{hsp18.2}::Gus* marker gene *Arabidopsis thaliana* plants⁶⁸ and during embryogenesis⁷⁴⁻⁷⁷. HSC70 is shown to express during vegetative and reproductive stages of tomato^{75,76}. HSPs did not express in germinating pollen and early imbibing embryos although both were very thermotolerant, the preformed HSPs may be playing a potential role in providing thermotolerance. HSC are stored in mature pollen⁷⁴ and seeds, probably to ensure survival in anticipation of potential heat stress. The involvement of HSPs in temperature stress tolerance and normal development has to be further tested.

HSP and crop productivity under stress

Crop productivity or grain yield is the result of a series of processes involving growth and development spread over the entire life span of the crop. These processes are supported by and regulate the various metabolic processes at the cellular level. Grain yield represents the dry matter partitioned towards grains and hence is directly related to the total dry matter accumulation by the crop. Dry matter accumulated over a period of time is related to the net photosynthesis rate and the total leaf area. High grain yield is the culmination of complementary relationship between 'source' (photosynthate availability) and 'sink' (grain no. × grain weight)⁷⁸.

High temperature stress causes accelerated plant development and consequently reduces both vegetative growth and grain yield⁷⁹. At the cellular level, heat stress results in metabolic disturbances, depletion of

respiratory substrates and reduction of photosynthetic activity. It may also cause denaturation of proteins, inactivation of enzymes and damage to cellular structures. Heat stress is especially deleterious during grain filling stage when it inhibits starch accumulation leading to grain weight decrease⁸⁰.

Breeding high temperature-tolerant varieties of crops is an important component of breeding programmes. Stability in grain yield in stress environment is an acceptable criterion for expressing the relative thermotolerance of varieties and species. Stability analysis helps in identifying contrasting genotypes and species which provide ideal material for analysing the basis of thermotolerance at various levels of organization. Infact the response of plants to high temperature has been identified as a two-tier response. For a temperate crop such as wheat, increasing temperatures in the range of 18–32°C constitute high temperature stress while temperatures above 32–40°C constitute the heat shock range. The two ranges of temperature evoke distinct responses which differ considerably.

Although the deleterious effects of heat stress on wheat productivity were known and emphasized in the beginning of the century^{1,81}, in India and Australia, emphasis on understanding the mechanism underlying heat tolerance is only recent. It is realized that a complex character like heat tolerance with respect to grain yield may not be linked to a single metabolic process. Plants have a multitude of mechanisms which help them to survive and propagate under high temperature stress. These include heat stress avoidance and heat tolerance mechanisms.

The heat avoidance mechanisms enable the plants to keep their temperature lower than ambient, through mechanisms such as

- 1) Transpirational cooling (in spring wheat genotypes, canopy temperature depression was significantly and positively correlated with yield stability under unirrigated conditions⁸²).
- 2) Differences in reflection of solar radiation through increase in leaf hairiness and wax deposition.
- 3) Leaf shading of tissues that are sensitive to sun burn.

The heat tolerance mechanisms operate in situations when tissue temperature is higher and yet plant functions are maintained. These include

- 1) Biomembrane saturation^{83,84}.
- 2) Synthesis of enzymes and isozymes with broad thermal kinetic windows and protective enzymes such as glutathion reductase, peroxidase, catalase, super oxide dismutase, etc.
- 3) Protection of biomembrances, molecules, organelles and maintaining their function, where HSPs may play a very crucial role.

Over 7 million ha of wheat cultivated in the subtropics suffers from heat stress. In central and southern parts of India, wheat suffers from heat stress at both the ends of crop growth. Sorghum and pearl millet also suffer in Rajasthan where during seed germination the soil temperature ranges from 50 to 60°C. High temperature stress causes yield reduction through accelerated phasic development, accelerated senescence, reduction in photosynthesis, increase in respiration (maintenance respiration) and inhibition of metabolic process of grain development such as starch synthesis. Rice also suffers from high temperature stress in tropical areas. When temperature rises from 24°C to 28°C, quantum efficiency of photosynthesis is decreased by 5%, while the rate of respiration increased by 30%. Thus the dark respiration is a primary limiting factor for energy fixation by canopies in tropical rice cultivation.

The amount of solar energy harvested during crop growth depends on the leaf area, which depends on the proper germination, seedling establishment and tillering/branching. High temperature stress drastically reduced germination in wheat^{34,85}, sorghum, pearl millet³⁸ and maize⁸⁶. In all these crops, thermotolerance of germination and seedling growth, and the kinetics of HSP synthesis were positively correlated^{33,34,36,38,41}. In cereals, leaf and shoot growth occurs from meristems situated near the soil surface and thus high soil surface temperature may have adverse effect on tiller and leaf production. In Central India, where soil temperature reaches 35°C to 40°C at the time of sowing, wheat variety Hindi 62 performs better than high yielding varieties because of its ability to germinate under heat stress and maintenance of tiller production. Hindi 62 which has the capacity to germinate at high temperatures exhibits high amylase activity at 30°C compared to a susceptible variety⁸⁷. Also, in seedling stage Hindi 62 showed higher and faster accumulation of hsp16.9, hsp17.3 and hsp26.4 transcripts at 35, 40 and 45°C compared to susceptible varieties (Viswanathan and Khanna-Chopra, unpublished). Thus, it seems logical to conclude that HSPs may be an important component of stress tolerance during germination and seedling establishment.

After seedling establishment the biomass accumulation depends on two important processes, i.e. i) photosynthesis and ii) respiration (growth and maintenance). Photosynthesis is highly susceptible to high temperature stress. Photosynthate availability decides sink size and in turn crop yield. In C₃ plants, quantum yield decreased by 22% when the temperature increased from 20°C to 35°C. Net photosynthesis in wheat started to decline beyond 28°C. In photosynthesis PS II is the most susceptible component to high temperature stress⁸⁸. Heat shock induced integration of 22–25 kDa HSPs into thylakoid membranes in pea and localized onto the stroma of chloroplast^{3,29}. In *Chlamydomonas* HSPs 22 and 29 kDa were localized into grana lamellae during heat

stress and protected Photosystem II from photoinhibition⁴⁷. Rubisco, the most abundant protein on earth, is also susceptible to high temperatures. In wheat the synthesis of Rubisco SSU is inhibited at 34°C in the susceptible wheat cv. Sturdy, while cv. Mustang was able to maintain Rubisco SSU synthesis and was correlated with synthesis of unique HSPs⁴⁰. Although in higher plants, existence of several chloroplast-specific HSPs has been demonstrated in *Pisum sativum*, *Phaseolus vulgaris*, *Arabidopsis thaliana*, *Vigna sinensis*, *Zea mays* and wheat, their correlation with protection of photosynthesis has yet to be demonstrated. Thus, HSPs may be an important component of thermotolerance of photochemical as well as biochemical components of photosynthesis. Under high temperature stress, cells need more energy to protect/repair the heat damaged macromolecules, biomembranes, organelles and for acclimation/adaptive reactions, i.e. the maintenance respiration need will be more under heat stress. Heat stress drastically reduces mitochondrial respiration. This may lead to metabolic aberrations, even if it happens for 2–3 hours in the midday, and to low crop productivity. *Phaseolus acutifolius* had maintained its mitochondrial efficiency at 32°C and thus plant growth, while *P. vulgaris* did not maintain its mitochondrial efficiency and thus reduced plant growth⁸⁹. The direct correlation between HSPs synthesis, its localization in mitochondria and maintenance of mitochondrial efficiency at 42.5°C had been demonstrated in soybean seedlings⁴⁸. Thus, HSPs appear to play a vital role in protecting mitochondrial respiration of crop plants.

At molecular level by protecting and repairing the macromolecules (enzymes, carrier proteins, ion channels, etc.) and by targetting the denatured macromolecules for proteolysis, HSPs may play a vital role in thermotolerance of all the metabolic processes, throughout the crop growth period. Increased solute leakage is another detrimental effect caused by heat stress at organelle and cellular levels. Under heat stress the membrane thermostability is an important component of thermotolerance and is highly correlated with yield stability⁸². By selecting genotypes for high membrane thermostability, yield increase had been achieved under heat stress⁹⁰. Increased solute leakage is attributable to loss of membrane integrity through lipid phase transitions and the effect on membrane proteins. Leakage of substances (amino acids, sugars, ions) into the incubation medium from soybean seedlings at 45°C was prevented if the seedlings were pretreated at 40°C, 2 h and during this process a 15 kDa HSP associated with the plasmamembrane, appeared to play a role in the protection of membrane proteins during heat stress⁹¹. The other component of membrane thermostability is through membrane lipid saturation, relatively a long-term adaptive process. Therefore, it appears logical to conclude that HSPs may be one of the essential compo-

nents of thermotolerance mechanism of crop plants and crop productivity under heat stress. If HSP is to be used as a selection criterion in breeding for thermotolerance, its genetics and heritability must be known. Efforts to link HSP accumulation with QTLs have not given very promising results⁹². Hence more studies are needed in this direction.

Future prospects

The role of HSPs in thermotolerance has been questioned in higher plants^{93,94}, yeast⁹⁵ and *E. coli*⁹⁶. Plants have at least six *hsp* families with several members in each family. These *hsps* show differential expression under stress and development, hence all the HSPs may not be required for stress tolerance in all the tissues. Can thermotolerance of crop plants be increased by altering the *hsp* expression? Studies to answer this question are stymied because the following questions also remain to be answered.

1. Identification and assignment of the role in stress tolerance of each HSP *in vivo*.
2. In stressed cells, what decides the damaged protein to choose the salvage or proteolysis pathway?
3. How is the expression/action of HSF controlled under stress and development?

To engineer plants with temperature stress tolerance, *hsp* expression may become an important approach along with alteration in thermal kinetic windows of key enzymes⁹⁷ and membrane lipid unsaturation. Further, HSPs can be used as a selection criterion in breeding programmes aimed at thermotolerance.

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Slower-chemical or faster-electrical signalling under stress in plants: Is it the hare and tortoise story of a slower signal winning the race?

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In stress physiology, one of the controversies related to root to shoot communication under stress, has been whether electrical signals from roots precede the chemical signal, represented by the predominant positive signal, abscisic acid (ABA) which accumulates up to 50 fold in the roots and xylem sap of stressed plants. Electric signals can be produced and transmitted to the shoots 18 cm away from the roots in 25 s when an osmotic stress is given to the roots. However a recent finding that ABA applied to the roots itself can generate electrical signals has only fuelled or exacerbated the controversy. In this paper

we have attempted to analyse the relative merits of a faster but apparently short distance intense signal, with the slower chemical signals. We have critically assessed what appears to be a 'deliberate strategy' of the plants to spatially separate two diverse but equally effective signals. The question we pose in this paper is, can a chemical signal still precede an electrical signal? If true, the plant must devise a different way to release an already available sequestered chemical signal. This is akin to resolving the classical dilemma of what comes first the chicken or the egg.

MAN has been concerned with plant stress adaptations since the first pre-historic cave dweller selected seed for propagation from plants that performed better than their neighbours. Physical and biochemical responses of plants to environmental stresses have been studied for over a century and a great mass of data is available. These responses embrace a fascinating spectrum of adaptation, ranging from the survival of the unicellular

algae *Dunaliella* in the harsh saline waters of the dead sea of Israel through a process called osmoregulation¹, to the survival of *Opuntia*, the common cactus, in the Californian desert when the temperature of its shoot reaches 65°C, i.e. 17° above the air temperature².

Although these two examples represent plant adaptations to a saline and high temperature stress respectively, the predominant abiotic stress affecting plant growth and development is by far drought or water deficits. This concern is reflected by the number of

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books devoted exclusively to the review of plant adaptations to water deficit³⁻⁶. These adaptations are classified as drought avoidance or escape, dehydration postponement, and dehydration tolerance. In the second category, the study of stomatal behaviour under stress should rank first amongst all the traits which have been studied during the last four decades particularly after the advent of porometry (a system designed to determine the stomatal resistance to gas exchange as a reflection of the rate of water loss from leaves) in the late sixties. As Rascke⁷, one of the pioneers in stomatal physiology, put it, 'Land plants are in a perpetual dilemma throughout their lives: Assimilation of CO₂ from the atmosphere requires intensive gas exchange and the prevention of excessive water demands that gas exchange be kept low'. Plants have learnt through evolution to strike some compromise between these two opposing objectives. Inherent in this strategy of compromise is to develop a system of communication, which will signal the stomata to restrict opening to avoid excessive water loss. In this system a 'message' synthesized by the roots which is the first to sense the water deficit, leaves the root, uses the xylem as a conduit, reaches the stomata and restricts water loss as a classical 'first line of defence'.

In the last decade there has been considerable interest in this novel signalling mechanism in different stresses; drought flooding and saline stresses⁸⁻¹⁰.

Several investigators including the authors of this paper, have been interested in root to shoot signalling under water stress. These signals can be either the slower chemical signal or the amazingly fast electrical signals. Recently this novel root to shoot communication has found a place in the second category of drought resistance mechanisms, that is those associated with postponement of stress¹¹ because these mechanisms are primarily meant to be a 'first line of defence' against stress. Thus a new area of plant stress research has emerged, targeting distress signal communication in plants. Whichever the signal, chemical or electrical, the consensus reached was that using this communication system, the shoots system of the plants have found an incredibly simple way to 'sense' perturbations in the soil environment, well before the shoot itself experienced it. However no consensus has been reached on the basic issue of which signal is more effective; the electrical (also called action potentials) signal or the chemical signal.

The latter has received more attention if the number of reviews during the last five years is any indication¹²⁻¹⁵. The exponents of the chemical signal have essentially used three systems

(a) Split-root systems, with half the roots in wet soil and the other half in dry soil¹⁶.

(b) Long soil columns with the upper roots in the top dry soil and the lower roots in wet soil, initially¹⁷.

(c) A balancing pressure given to the entire root to counter balance the decreasing water potential of shoots¹⁸.

All these had one thing in common, i.e. the whole root or a part of the root system was in dry soil, while the water status of the shoots was similar to that of non-stressed controls. Any change in a physiological process (either stomatal conductance or leaf expansion) can therefore be directly attributed to the production of a chemical signal produced by the loss of turgor of that part of the root system in dry soil. This signal was transported to the xylem and subsequently sent up to the epidermis of the leaf. Immunological quantification of the signal in the epidermis has shown convincingly that it is the signal arriving at the epidermis from the roots which causes the initial restriction of the stomatal opening¹⁶. This early warning signal coming from just a few roots which are the first to dry (in the natural field situation the top 10-15 cm of the soil profile is also the first to dry) is called the 'first line of defence'¹⁹ as it helps to conserve water by reducing transpiration (restricting stomatal opening) and transpiration area (decrease in leaf expansion) so that some water is available at later stages of growth. Such a 'strategy' therefore enables the plant to balance its size with the availability of water.

The nature of chemical signal has received a lot of attention and has been reviewed thrice¹³⁻¹⁵ during the last three years, with the stress hormone ABA receiving primary attention as the predominant positive or accumulating signal. Levels of this hormone have been shown to increase up to 50 fold in the xylem sap¹⁹⁻²¹, enroute to the shoots.

The electric or action potential has received more attention *vis-à-vis* the 'touch me not' syndrome in *mimosa*^{22,23} since the pioneering work of Sir J. C. Bose²⁴ in 1913. Only recently were elegant experiments conducted to show that these faster signals (speed of transmission 7-14 mm/s) can be equally effective as a 'first line of defence' in distress signal communication under stress. Hebbar *et al.*²⁵ were able to show quite convincingly that when a sudden or snap osmotic stress was applied to the roots of sunflower, an electric signal originating in the roots reached the shoot in 25 s and manifested itself ultimately as a decrease in the stomatal conductance (the first line of defense). In another recent work, action potential was also shown to be involved in wound-induced signalling system of plants²⁶. But taking the electric signalling work of Hebbar *et al.*²⁵ (Figure 1) under osmotic stress as a representative example and comparing it with any one of the examples of chemical signals; (say that of the Zhang and the Davies group¹⁹ or our own work²⁰), one realizes that it becomes extremely difficult to analyse the relative merits of either of these signals because of the following reasons.

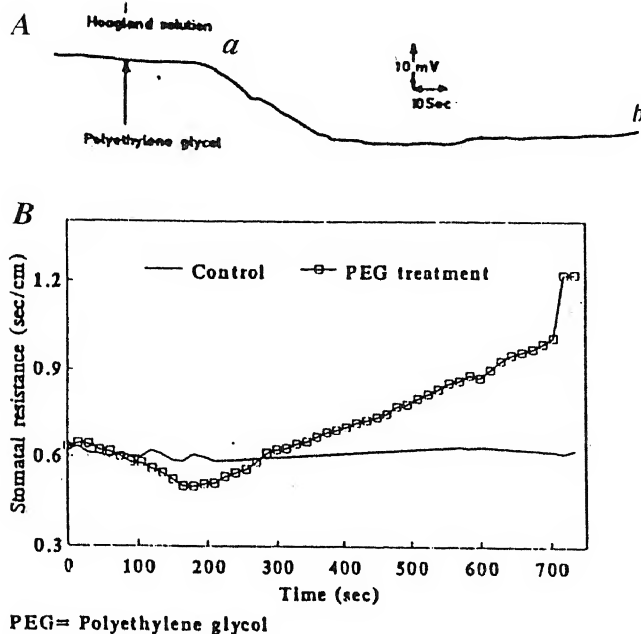


Figure 1. Influence of action or electrical potentials on stomatal or physiological processes under osmotic stress. (A) Effect of osmotic stress on surface electrical potential at the shoot apex of sunflower seedling. *a* = pattern of electric potential in control, *b* = pattern of electrical potential in response to osmotic stress. (B) Changes in stomatal resistance in control and PEG (25% w/v in Hoagland solution) treated sunflower seedlings with time. (after Hebbar *et al.*²⁵)

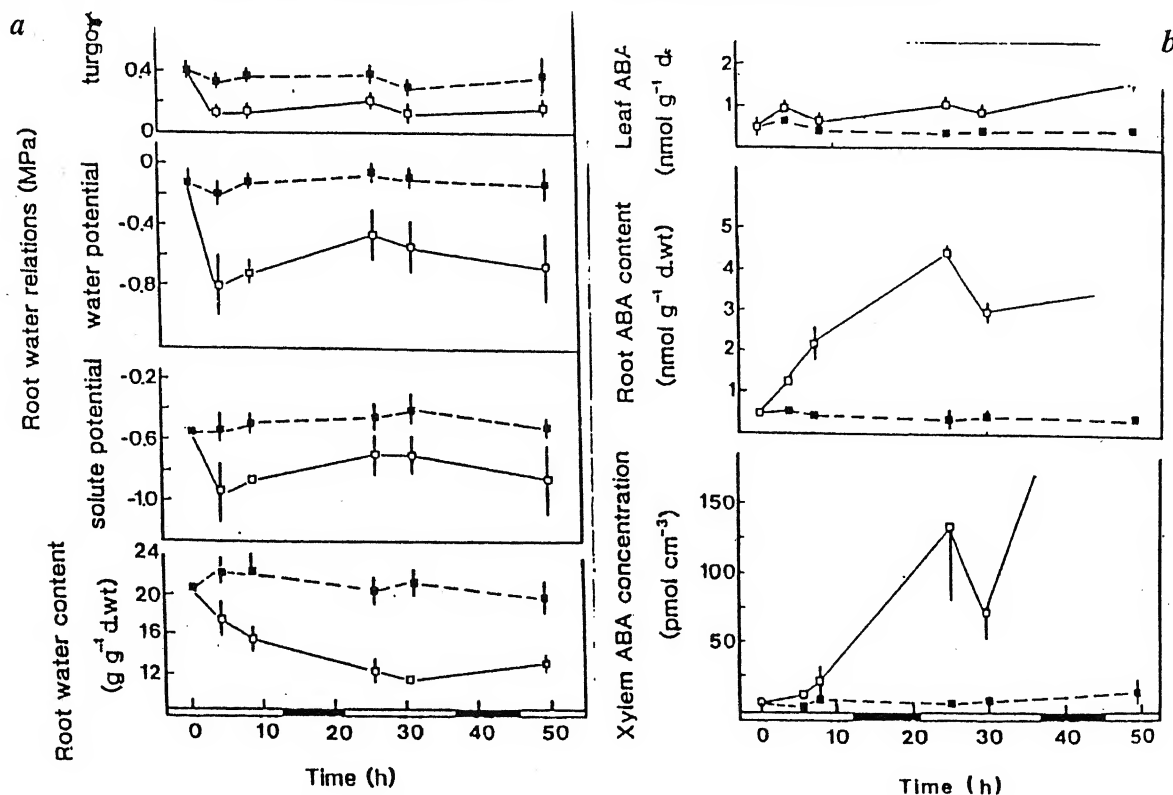


Figure 2 *a, b*. Influence of chemical signal on physiological process under water deficits simulated in the vertical split root system. *a*, Water relations of roots of *Helianthus* plants which protrude from soil, through the base of the pot. Roots in the nutrient solution after time 0 (controls, ■). Roots in moist air after a brief period of air drying (treatment, □). Each point is the mean ($n = 3$) value with \pm s.d. bars. *b*, The ABA concentration in leaves, roots and xylem sap of control *Helianthus* plants (■) and in plants where protruding roots were dried (treatment, □). Points are means of $3 \pm$ s.d. Root data shown are for protruding roots. (after Neales *et al.*¹⁹)

(a) Hebbar's work involved a drastic osmotic stress, and therefore would be expected to convey the action potential in a few seconds.

(b) Our own work reported earlier or that of the Davies group involves a slow dehydration of the roots either naturally in a soil column or an artificial one where the lower, free hanging roots are allowed to lose water. This process even if accelerated involves a time frame of hours. For example, Neales *et al.*¹⁹ utilizing this system showed a decrease in conductance of 30–40% in maize plants through a chemical signal ABA, in 5 h, by slow dehydration of the lower free hanging roots (Figure 2).

In all these cases, the root–water relations are mandatory (see Figure 2 of Neales *et al.*, 1989) and is useful to compare the extent of the ABA signal from different laboratories. A PEG stress, on the other hand, besides being a drastic stress has several shortcomings.

(a) Water relations of the root is extremely difficult to determine.

(b) The roots attain equilibrium with the water potential of the osmoticum only after several hours.

(c) Long before that the action potential has already been transmitted to the shoot.

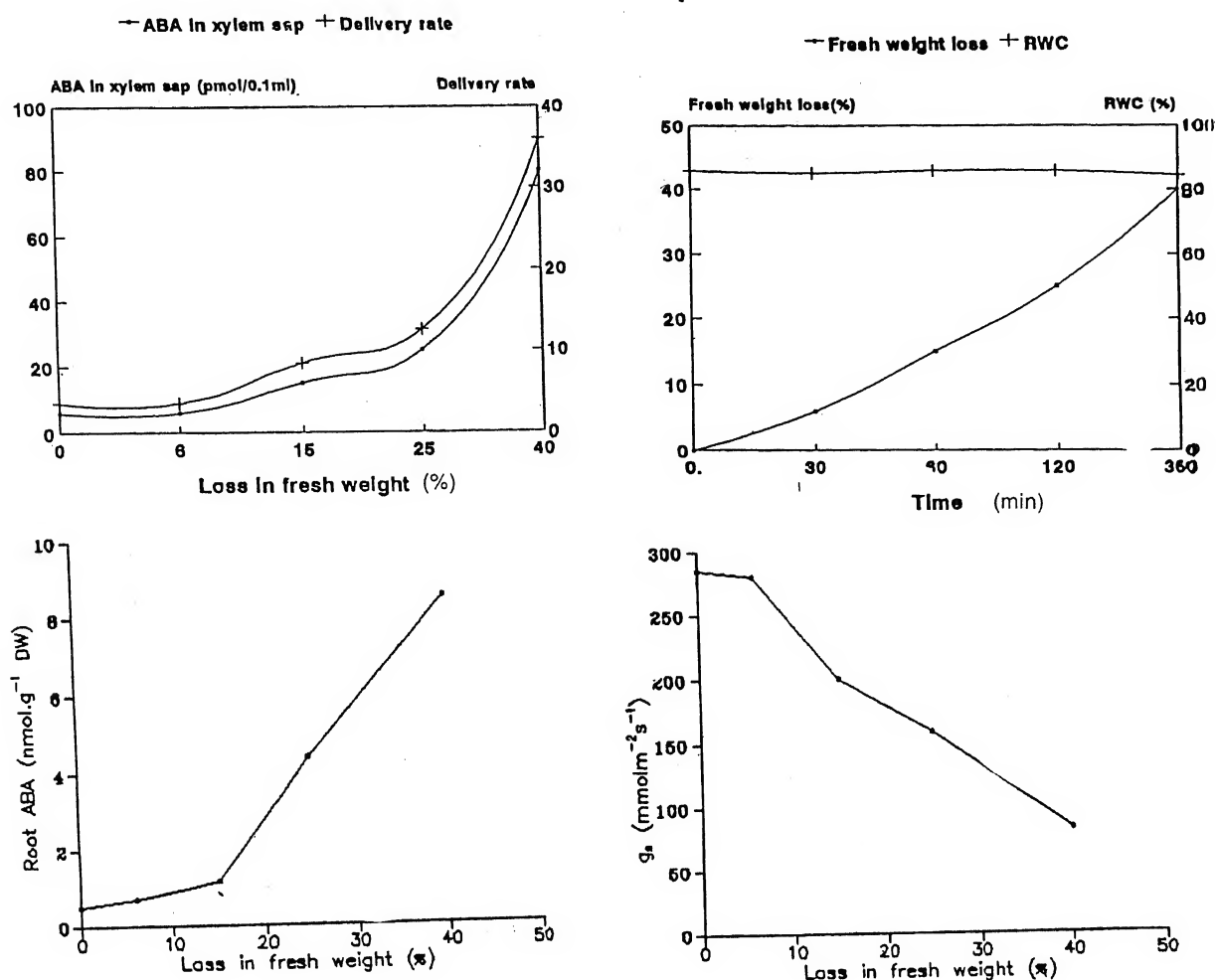


Figure 3. Changes in water relation, stomatal conductance and chemical signal ABA on rapid dehydration of free hanging roots in a vertical split system.



Figure 4. The vertical split root system developed by the Zhang and Davies group¹⁶ (upper roots grow in wet soil while lower roots protrude through the base of the pot and hang freely. These roots can be dehydrated either rapidly (outdoors) or slowly (suspended in a humid cabinet).

(d) Comparisons between work of different workers are near impossible as equilibrium time varies with the morphology of the roots of different species.

How then does one analyse the relative merits of an electric signal and a chemical signal? The fact that the former is faster is obvious. Why then does a plant spend so much energy on producing a second chemical signal? The answer could hinge on resolving an entirely different issue.

(a) Is an electric signal only capable of being transmitted up to short distances (most work on these signals deals with distances of 18–20 cm, rarely more)? In fact Zawadzki²⁷ clearly showed the limitations of an electric signal with regard to the distance. The action potential, he observed, has three major limitations.

(a) Occurs only when the stem was electrically stimulated, not the leaves and roots,

(b) Does not spread to the neighbouring internode,

(c) Gets extinguished in the region of the stem apex and root neck.

The first one of course has been quite convincingly disproved by Hebbar's work, but the second and third suggest that an electric signal could fulfill only the initial need of transmitting a fast signal up to a short distance. Later a greater more intense long range signalling mechanism could operate (a chemical signal has also been shown to operate in the tree seedlings of apple²⁸).

Resolving this issue has, however, become more complicated by the recent observation of Fromm²⁹ that the application of ABA (a chemical signal) was itself able to elicit an electric signal that travelled to the leaves within 2.5 seconds. The classical dilemma is what comes first – the chicken or the egg or to use a more scientific jargon, can a chemical signal precede an electrical signal. But since ABA accumulation has been shown to require transcription³⁰, it is obvious that a chemical signal cannot be visualized in a time frame of seconds but only in hours. Very recently we specifically conducted experiments to resolve at least partly this question, i.e. can a time frame for a chemical signal to be sent from roots to shoot, be reduced at least to minutes. We fixed 30 min as the minimum time required for ABA accumulation after transcription and translation and artificially accelerated the process of dehydration of the roots using the split root system developed by Zhang and Davies¹⁶ (Figures 3 and 4). When the lower free-hanging roots were dehydrated quickly and made to lose 6% of their fresh weight in less than 30 minutes, ABA increase in roots and xylem sap was still not significantly different from the control levels at 0 hours. Only a 15% loss in fresh weight in 1 h caused a significant increase in ABA which apparently in turn caused a significant decrease in conductance (Figure 3). Our data corroborates closely with that of Neales *et al.*¹⁹ who also showed that a 20% drop in root water content in 5 h resulted in a significant increase in ABA. What we have done is merely shifted the time frame to 1 h instead of the 5 h by accelerating the process of dehydration. Once the required threshold loss in turgor is achieved, then current models are adequate to explain the mechanism of accumulation at the cellular and molecular level³¹.

It is impossible to visualize a time frame of seconds for a chemical signal like ABA to be detected in the roots even if it was caused merely by release of already sequestered ABA from organelles³² due to ion gradients and not ABA synthesis. Since the latter requires transcription, one would have to visualize a time frame of at least 15–30 minutes for synthesis of ABA, even if one were to accelerate the dehydration process by whatever means to make the roots reach the threshold loss of turgor in just a few minutes.

Keeping aside these arguments, how does one explain Fromm's observation of an electrical signal being produced by ABA application to the roots in seconds. Clearly more work needs to be done at cellular levels using sophisticated neurophysiological techniques like

the patch clamp, while giving credit to Sir J. C. Bose for pioneering its use in the beginning of this century, exactly eighty-three years ago. Until then we would have to be satisfied merely by disagreeing with the moral of the hare and tortoise story, i.e. there is no way the tortoise (chemical signal) can win. The hare (electrical signal) will win all the time.

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RESEARCH ACCOUNT

Application of ion–solvent interaction parameter in interpreting the kinetic profiles of Diels–Alder reactions and thermal stability of DNA duplexes in ionic solutions

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Rate enhancement in Diels–Alder reactions and thermal stability of a DNA duplex at several ionic concentrations are the main issues addressed in this account. The proposed quantity, internal pressure of a salt–solvent, which is also a measure of electrostriction effect together with the activation volumes of reactions can describe the impressive rate accelerations of the Diels–Alder reactions carried out in salt solutions. Similarly, obviation of high pressure conditions for the synthesis of organic molecules is described. More importantly, the application of the concept of internal pressure and volume has demonstrated that the thermal stability of several DNA duplexes in the presence of ions can be explained. The pressure dependence of thermal stabilities at constant ionic concentrations is linear in nature. The correlations exposed for the DNA duplexes have strong potential to unravel the forces responsible in stabilizing a DNA duplex or the formation of a duplex from its complementary strands.

MOLECULAR interactions of a solvent molecule with nonionic and ionic solutes play vital roles in governing the static and dynamic behaviour of a system. Various thermodynamic, kinetic and other solution properties of the system represent specific behaviour of these molecules in a given environment. With regard to solvents,

Mother Nature discovered the secrets of water in different biological and other natural processes, whereas nonaqueous solvents continued to gain importance in a variety of man-made processes.

Considering the importance of the solute–solvent interactions in several processes, we have very recently embarked on a programme to investigate their role in controlling the kinetic profiles of the organic reactions, like Diels–Alder (D–A) reactions^{1,2} and the association and dissociation processes involved in nucleic acids³. Some of these results along with the futuristic views have been described here.

Definition of solute–solvent interactions in the present context

Cohesion among molecules in the liquid phase results from intermolecular forces. These forces, in general, include hydrogen bonding, dipole–dipole, multipolar, dispersion interactions and also interactions emerging from the repulsion between two molecules. The cohesion due to intermolecular forces gives rise to a 'pressure' which is experienced by the solvent molecules. This term for pure solvent was initially proposed by Hildebrand and Scott⁴ and subsequently supported by the equation of state⁵. A liquid undergoing a small, iso-

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thermal volume expansion does work against the cohesive forces which causes a change in the internal energy, U . The function $(\partial U/\partial V)_T$ is called internal pressure of a liquid. According to Hildebrand and Scott⁴, definition of internal pressure for a non-polar liquid can be transformed into that for polar by a specific factor indicating the polarity of a solvent. Thus, the internal pressure for a non-polar liquid differs from that of a polar. For instance, the internal pressure values for water and *n*-hexane are noted to be 166.4 MPa and 235.7 MPa, respectively.

Internal pressure changes upon the addition of a solute. Such addition of a solute, particularly of ionic nature, brings about a great change in the cohesion of a solvent. While some solutes like NaCl, KCl, etc. enhance the internal pressure of the solvent, salts like of guanidinium decrease it. As seen later, this phenomenon in solution can prove to be a vital tool in unraveling the origins of forces involved in some organic reactions and more importantly, the thermal stabilities of nucleic acids.

Due to the high electric field associated with an ion, water molecules are oriented around it, causing a local collapse of the bulk water structure. Since water molecules are more firmly packed around an ion than in bulk water, the net volume of the system decreases in the region. This process, called electrostriction (ES), is thus a result of ion-water interactions and it mainly depends on the charge and the radius of an ion, and the changes in local dielectric constant around the ion^{6,7}. The ES effect can be described in terms of internal pressure. Internal pressure, P_i of a solution can be estimated from the experimental quantities by the expression $P_i = \alpha T/\beta$; where α and β are the coefficient of thermal expansion and isothermal compressibility, respectively at a temperature T . Both α and β can be measured from experiments on volumetric properties. In the cases where these values are not readily available, the scaled particle theory⁸ can be used. We have employed this theory for the solute and solvent of different sizes⁹. This theory yields P_i to within $\pm 3\%$, when compared to experimental data. In Table 1, the P_i values for some salts in water are represented by a polynomial equation as a function of the salt concentration, M .

Fascinating observations in the kinetic profiles of D-A reactions

These addition reactions result from the reaction of a diene with dienophile and are useful in synthesizing complex organic molecules. Several years ago, Rideout and Breslow¹⁰ demonstrated the application of pure water as a reaction medium in these reactions. Two exciting outcome of this medium were the impressive enhancement in reaction rates by several order of magni-

Table 1. Parameters of the equation $P_i/\text{MPa} = 166.4 + aM + bM^2 + cM^3$ for a few representative salts in water ($P_{iw} = 166.4$ MPa) as function of M (mole l^{-1})

Salts	a	b	c
NaCl	10.792	61.273	0.532
KCl	18.841	70.541	-0.096
NaClO ₄	88.114	-1.565	0.011
KCNS	90.923	-1.734	0.011
CF ₃ COONa	86.464	-1.516	0.007

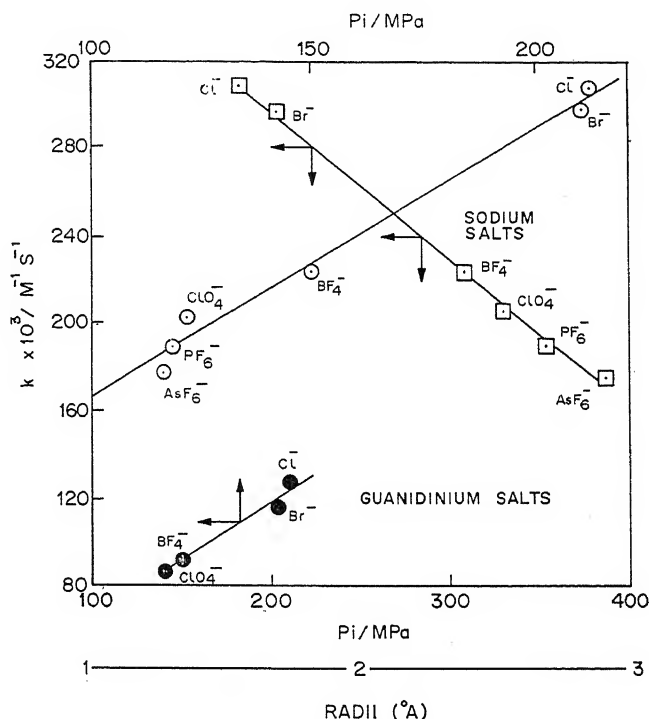


Figure 1. Reaction rates k versus internal pressure P_i (○, ●) for reaction of *N*-ethylmaleimide with anthracene-9-carbinol in salt solutions of sodium and guanidinium; analogous plots of k versus anionic radii (□) are also included.

tudes in the cases where the reaction in other solvents was either not possible or very sluggish. Secondly, the stereoselectivities in the reaction products (endo/exo ratios) could be altered to some extent. Success of the reactions in water prompted the application of 'solute-solvent' additives for altering the kinetic profiles of some very cumbersome reactions. One of these reactions is the synthesis of cantharidin, which was conventionally synthesized under an external ultrahigh pressure of 16–18 kbar or 1.6–1.8 GPa (ref. 11). The use of a reaction medium comprising of lithium perchlorate-diethyl ether (LDPE) led to the synthesis of cantharidin with better yields at atmospheric pressure¹². Conversion of a high pressure reaction to an atmospheric reaction using such additives which are comprised of ionic solutes and water or nonaqueous solvent, proved to be a milestone in

synthetic chemistry. Following this work, several sluggish reactions have been successfully conducted in such media²⁻¹⁴. Similarly, the use of other salts like LiCl, NaCl, etc. in water has further enhanced the reaction rates. On the other hand, salts like guanidinium chloride have retarded the reaction rates. Figure 1 depicts a general picture of variation in the reaction rates of such D-A reactions in the pressure of salt-solvent systems. Examples of some D-A reactions in aqueous as well as in nonaqueous solvents with ionic solutes are mentioned in Table 2. Rate enhancement in aqueous media has been ascribed to several effects like hydrophobic interactions, micellar catalysis, hydrogen bonding and solvent effects. An excellent discussion on these effects has been recently documented¹³⁻¹⁴. One of the most potential explanations to describe the impressive rate enhancements is based upon the Lewis acid catalysis of the reaction.

In short, several other contrasting explanations to describe the origin of forces that are responsible for such effects have been put forward. With due credence to all the explanations, we in our recent work, have attempted to correlate and explain the variations in reaction rates with the help of a bulk property of solution, i.e. internal pressure as shown in the following discussion.

Possible explanations of the reaction profiles of the D-A reactions

First, we examine the reactions in aqueous reaction media. Two situations in this case are pertinent, where the reaction rates of the D-A reactions are increased or decreased by different salt-water systems¹³. Among others, a simple reaction of *N*-ethyl maleimide and anthracene-9-carbinol shows enhancement in rates in presence of aqueous NaCl, NaBr, etc.¹⁵. On the other hand, the rates are decreased by the medium of guanidinium salt. An examination of the P_i values in such salts shows that those reaction media which enhance the rates, also exhibit the increase in internal pressure. The same is true

for the decrease in the rate reactions. It is, thus, possible to correlate the rates with internal pressure for different reactions. In Figure 1 are shown the reaction rates versus P_i for the above reaction in the presence of sodium and guanidinium salts. An excellent linear plot is obtained in this system. Such linear plots between the reaction rates and internal pressures for several other reactions in aqueous environment indicate that the internal pressure can be used as a strong correlating parameter in these reactions. As a matter of fact, the arrangement of water molecules is drastically changed in the presence of salts¹⁶.

This change affects the transition state of a reaction. The increase in internal pressure with increasing concentration of salts like NaCl, NaBr, etc. enhances the ES effect. On the contrary, the ES effect due to the guanidinium salt type decreases with the increase in the ionic concentrations. An important outcome emerging out of such work is the possibility of the role of hydrophobicity¹³.

Both diene and dienophile due to their dislike for water come close enough to react. This hydrophobic effect is further enhanced in the presence of those salts which accelerate the D-A reactions. Obviously, a decrease in hydrophobicity can be considered, when the reaction rates decrease in the presence of salts like those of guanidinium. Similarly, the hydrophobicity increases in the rate enhancing salts¹⁷. We have also noted that the reaction rates also depend upon the size of salt used as a reaction medium. As seen in Figure 1, the reaction rates decrease, if the size of anions increases for the same salt series keeping the cation common. This variation is consistent with the increasing or decreasing hydrophobic effects in the presence of salts and their relations with the reaction rates. These results can also be understood in terms of ion-water interactions.

Though our primary objective in this account is focused on aqueous environment, it is essential to briefly mention ionic interactions in nonaqueous solvents and their implications on the reaction rates. Such interactions, like those available in the lithium salts with diethyl ether, acetone, ethyl acetate, etc. can be used to correlate both the reaction rates and stereoselectivities of the D-A reactions². For obtaining these correlations, activation volume and internal pressure P_i are used to describe the rate data.

Activation volume, an activation parameter is the only appropriate choice for studying a reaction, when pressure of the system is variable. Activation volume (ΔV^\ddagger) data yield valuable information on the kinetics of reaction. The ΔV^\ddagger is defined as the difference of reactant partial volumes and the volume of the transition state. The ΔV^\ddagger can be obtained by the use of liquid phase non-ideality calculations, besides high pressure kinetics¹⁸. In the method used in our work, activity coefficients were deployed to yield liquid phase non-idealities of compo-

Table 2. Some examples of the Diels-Alder reactions promoted by the salt-solvent systems

Reactions	Systems
1. <i>N</i> -ethylmaleimide + anthracene-9-carbinol	aq., aq. LiCl; GnCl*, LiClO ₄ ; GnClO ₄
2. 9,10-dimethylantracene + acrylonitrile	aq. LiCl
3. Isoprene + <i>N</i> -phenylmaleimide	aq. LiClO ₄
4. Cyclopentadiene + methyl vinyl ketone	aq. LiCl; aq. GnCl
5. 2,3-dimethylbutadiene	LiClO ₄ + acetone
6. Furan+ 2,5-dihydrothiophene-3,4-dicarboxylic anhydride	LiClO ₄ + diethyl ether

Gn* = Guanidinium

nents. We used a well-known model known as Universal Functional Group Contribution model abbreviated as UNIFAC¹⁹, where the interactions between cation, anion and their individual and mutual interaction with solvent are explicitly considered by a combination of extended Debye-Huckel equation and interaction energies of nonideality. In a nutshell, both quantities i.e. internal pressure and activation volume offer us excellent correlations with the kinetic profiles. Thus, it is possible to explain why the reaction rates vary with ionic concentrations and why two reactions show different reaction profiles under specific conditions of a reaction medium. In further work, we have also been able to demonstrate the utility of our proposed concept in dealing with the D-A reactions conducted in different pure solvents and their aqueous mixture²⁰. The parameter on which such correlations are based, requires the internal pressure normalized to a polarity scale.

In the foregoing discussion, we noted that the addition reactions like the D-A reaction, which yield impressive rate enhancement in the presence of salt solutions, can be described using an internal pressure term. In essence, enhanced internal pressure together with activation volume of a reaction prove to be of tremendous use in understanding the kinetic accelerations in the D-A reaction in the ionic solutions described above.

With this development in reaction kinetics, it was intriguing to explore the possibility of explaining melting behaviour in nucleic acids. Melting of a nucleic acid, for example, of DNA duplex involves a dissociation process or reaction, which takes place in ionic environment. On the other hand, the formation of a DNA duplex in ionic environment is an association reaction. In the following discussion we shall attempt to examine the dissociation or association reactions in a similar fashion that was used to correlate the kinetic data of Diels-Alder reactions.

Nucleic acids

Effect of various salts

Although both proteins and nucleic acids are particularly prominent among the molecules essential to life, molecular interactions in nucleic acids, particularly DNA will be the sole focus of our discussion. DNA is a very long biomacromolecule composed of a large number of deoxyribonucleotides. A nucleotide consists of three molecular fragments, i.e. sugar, base and phosphate. Interactions to nucleic acids are highly specific and play crucial roles in controlling the transcription, recombination, replication, etc. An important aspect of a DNA molecule which warrants attention is its stability in aqueous ionic media. This stability can be judged by the melting of DNA solution or adding some ionic sub-

stances. The melting temperature, T_m at which a DNA double helix converts into two complementary strands, depends markedly on the base composition, base stacking and the ionic environment in general²¹⁻²⁶. In fact the very formation of the DNA duplex from its complementary single strands is governed by these parameters. It is established both by experiments and theory that DNA molecules are heavily hydrated. Effect of water on the stabilization of DNA helices via hydration, therefore, assumes a vital role in understanding the energetics of DNA.

As a matter of fact, interactions of counter ion Na^+ with the regular lattice of negatively-charged phosphate groups (PO_4^-) of the sugar-phosphate backbone of the DNA helix can change the degree of hydration and contribute to the stability of DNA helices. A valuable contribution in this regard has been made by Manning²⁷, who used a rigorous approach of counterion condensation, where the statistically averaged properties of mobile cloud of counterions tends to neutralize the net electrostatic charge of the nucleic acid. Later, Record *et al.*²¹ utilized the polyion condensation theory of Manning for the analysis of stability of the DNA helices. In their simple treatment, they introduced a concept of thermodynamic binding, defined on the basis of a suitable combination of several ionic effects. These interactions which are electrostatic in nature have been the subject of intense investigation by theoretical methods. The classical methods of analysing these interactions have largely been based on the Debye-Huckel, Gouy-Chapman, Poisson, Poisson-Boltzmann equations, etc. whereas Monte Carlo simulations, molecular and Brownian dynamics are some of the very recent techniques used to understand the role of ions on the stability, structure, dynamics and related properties of DNA²⁸. The resultant equations have been useful in analysing the stability data of DNA helices particularly in the low ionic concentration range. A reference should be made to the work of Troll *et al.*²⁹ in which the effects of permittivity discontinuity at the boundary between aqueous phase and the low dielectric DNA cylinder were studied in order to understand the stability aspects of the DNA helices.

As seen above, several qualitative and quantitative explanations have been put forward to describe the stabilization of the DNA helix in aqueous medium. In our work, we have been primarily concerned to gain insights into the process of formation of a DNA helix from its complementary single strands or the melting of a DNA helix into two complementary single strands in the presence of an ionic atmosphere. For explaining the thermal stability effects, we have preferred to correlate T_m with the process of electrostriction. It seems to us that the electrostriction effects, if expressed in terms of a measurable thermodynamic quantity, can be used to interpret several features of the thermal stability of a

DNA helix, without recourse to much complex mathematics. A quantity useful in the present analysis of thermal stability of DNA helix is the volume change, ΔV , accompanying the transition process. The hydrated DNA consists of two discrete layers representing primary and secondary hydration shells. In fact, the bases in single strands tend to form complementary hydrogen bonding leading to the formation of hydrophobic core in the DNA helix. The phosphate groups lie exposed to water and impart considerable charge density to single strands and to their double helix. The magnitude and sign of ΔV depend upon the base stacking, the degree of hydration, the charge density parameters of both the single complementary strands and duplex, and the electrostriction of the water molecules^{23,24,26,30,31}.

Both the P_i and ΔV parameters can now be utilized to examine the issue of thermal stability of a DNA duplex in different situations.

Thermal stability – Enhancing behaviour

It is established that the thermal stability of the duplex is greatly influenced by salt concentrations²¹. For instance, higher concentrations of NaCl induce greater stabilization of the duplex (higher T_m), as compared to lower T_m noted at low concentrations data of salt. Our calculations of P_i using the most reliable density and compressibility^{32,33} show that P_i of water increases upon the addition of NaCl. Such a variation in P_i can be indicated by the effective internal pressure ΔP_i , as $\Delta P_i = P_i - P_i^w$ where P_i^w is internal pressure of pure water. The term P_i denotes the change in internal pressure of water occurring upon the addition of a salt. The aqueous ionic environment thus remains under high pressure conditions, which are enhanced almost up to the solubility limit of NaCl in water. The transition of a duplex to single strands is reported to be accompanied with the positive volume changes. The possible reasons for the positive ΔV values and their variations with the salt concentrations, observed in the above transition of a duplex can be understood in terms of electrostatic and stacking effects^{34–36}. For instance, in the case of Poly(dA).Poly(dT), the ΔV values are noted to vary from 2.60 to 7.81 cm³ mol⁻¹ in the range of NaCl concentration from 0.02 to 1 M. The ΔP_i value at 1 M is much higher than that at 0.02 M. This high internal pressure suppresses the process of transition of a DNA duplex to single strands since V is positive for the melting process. Thus, in order to obtain transitions at a higher NaCl concentration, a higher temperature needs to be employed. This leads to a higher T_m value which increases with the addition of NaCl. In other words, one may state that the ES or the enhanced P_i resulting from the salt concentrations controls the T_m of a duplex DNA. To illustrate these variations, the relationship between

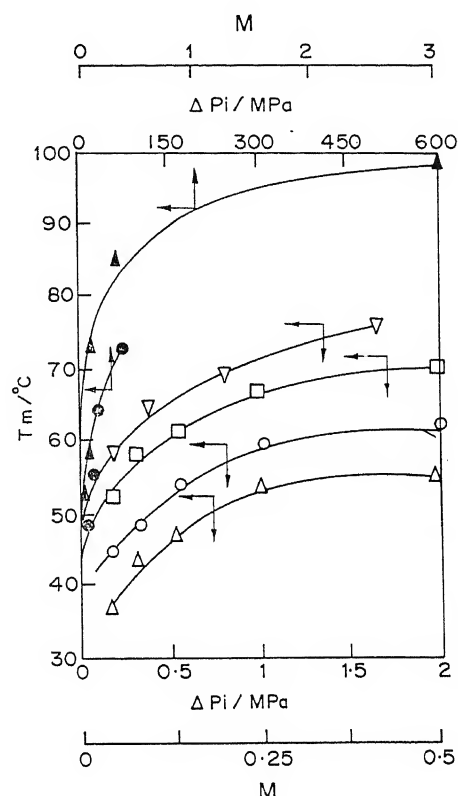


Figure 2. Relationship between transition temperature T_m and effective internal pressure ΔP_i for the double helix transition. ▲, Poly(dA).Poly(dT); ●, Poly[d(A-T)] Data ref. 36; ▽, Poly(dA).Poly(dT); □, Poly(rA) · Poly(dT); ○, Poly(rA).Poly(rU); △, Poly(rA).Poly(dU) (data Rentzeperis *et al.*²⁵). First data set at high NaCl concentration; All other sets low NaCl range; corresponding salt concentrations are also shown.

T_m and ΔP_i are shown, in Figure 2 for the transitions of several duplexes of Poly(dA).Poly(dT) or the polymers containing AU base pairs in different NaCl concentrations, ranging from low to high, at atmospheric pressure. The duplexes with GC base pairs are known to have higher T_m values owing to lesser hydration and more number of hydrogen bonds as compared to the AT base pairs. In the case of duplexes³⁷ with high GC contents, curves of T_m versus P_i are shown in Figure 3. The shape of the curves is a typical temperature–pressure plot encountered in transitional reaction kinetics. In the case of higher valent salts like MgCl₂, higher values of P_i are obtained as compared to those for NaCl at corresponding concentrations. In general, values of P_i for MgCl₂ are almost three times as high as those for NaCl up to 4 M, as calculated from literature data^{32,33}. Thus, almost the same stability for a given duplex can be obtained using a much lesser concentration of MgCl₂ as compared to a given concentration of NaCl^{21,38–41}. The ES effect enhances when one moves from uni-, multi-valent ionic salts¹⁸ for which the thermodynamic support comes from the activity coefficient data⁴².

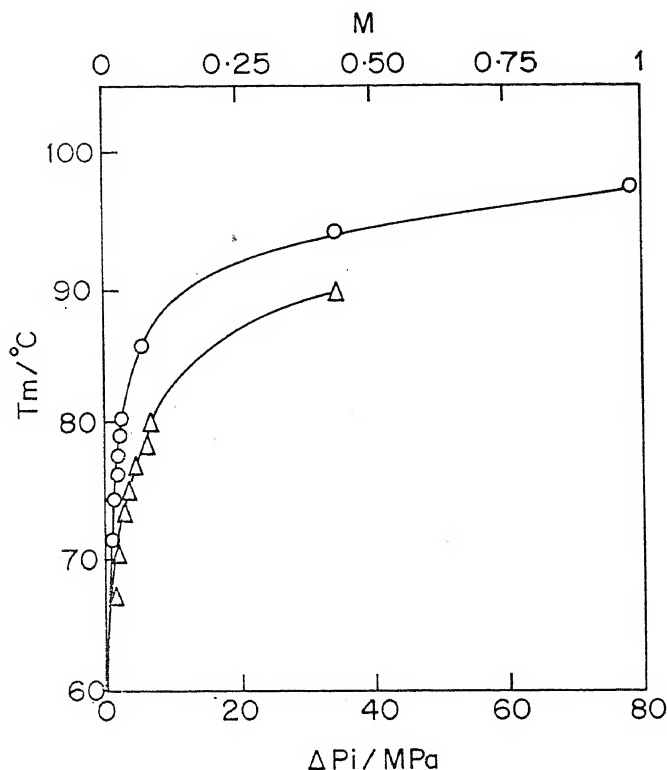


Figure 3. Demonstration of relationship between T_m and ΔP_i for the denaturation of *E. coli* (O) and *D. pneumoniae* (Δ) with high GC contents in KCl. The corresponding concentrations are given in ref. 37.

Thermal stability decreasing behaviour

As seen above, thermal stability of a DNA duplex increases by the addition of certain salts like NaCl, KCl, etc. Contrary to the observation, the DNA duplex is destabilized in the presence of some other salts, such as acetates, perchlorates, etc. As a matter of fact, it is the property of a salt or ionic media, which has a strong correlation with the variations in T_m of a duplex. For instance, the T_m values of a duplex in the presence of NaClO_4 , KCNS and CF_3COONa at several ionic concentrations are reported to be decreasing with increasing concentration⁴³. Explanation of this experimental observation can be found out using the concept of negative changes in ΔP_i upon the addition of salts. Values of ΔP_i in such salts can be calculated from the experimental data or theoretical methods. Such cases of decreasing T_m with increasing salt concentrations is demonstrated in Figure 4, where the T_m values for these salts are plotted as a function of ΔP_i . There are two interesting aspects in this case; (i) the ΔP_i values are negative and (ii) the curves are smooth up to high concentrations of salts. An examination of plots reveals that the proposed correlation is applicable even to a situation where the T_m decreased by 60°C. The ΔP_i values for CF_3COONa

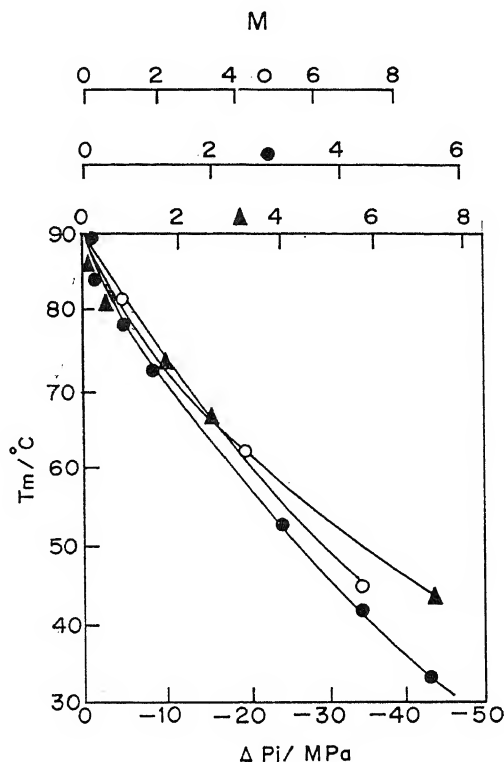


Figure 4. Plot of T_m against ΔP_i for denaturation of DNA duplex in the presence of salts. \blacktriangle , NaClO_4 ; O, KCNS; \bullet , CF_3COONa (ref. 47).

demonstrate the salt's capability as the most powerful denaturant out of the three studied. In such cases, the negative, ΔP_i values are the results of the anti-electrostriction effect caused by salts as indicated above. In other words, the difference between the molar volume of a liquid salt, and its partial molar volume tends to become negative. This situation appears to be analogous to one arising out of the salting-in effect. Salts like those shown herein have very large anions that make the charge density smaller than that of water dipole, and thus decreasing the interactions as compared to those among water molecules. This argument is supported by several theoretical calculations⁴⁴.

Both the thermal expansion and isothermal compressibility, i.e. α and β , respectively are responsible for calculating P_i values of a system. In the above examples both the contrasting cases of T_m -enhancing and reducing thus should be examined in terms of the α and β terms. It was noted that the salts which enhance the thermal stability with increasing concentration, exhibit a noticeable increase in the thermal expansion and a decrease in the isothermal compressibility with an increase in salt concentrations. On the other hand, for the T_m decreasing salts, an opposite trend is observed, i.e. an increase in isothermal compressibility dominates the thermal expansion when one moves from a dilute to a concentrated

salt solution. For these salts, one can think that a situation of internal pressure drop over pure water is created due to ion-solvent interactions, which when coupled with positive ΔV values, tends to destabilize the duplex, with the consequent lowering of T_m . This destabilizing effect continues to dominate with the increasing salt concentration. The anionic size in the salts influences the internal pressure drop in such situations.

The T_m values for different duplexes at the same ionic concentration show variations, which can be analysed on the basis of similar arguments. Since the ΔV values for the duplex depend upon several parameters described earlier, the effect of a given NaCl concentration or the thermodynamic internal pressure can easily explain the differences in T_m values for the different duplexes. For example, the ΔV values for the transitions of Poly(dA).Poly(dT) and Poly[d(A-T)] at 0.2 M NaCl concentration are 4.59 and 2.14 $\text{Cm}^3\text{mol}^{-1}$, respectively³⁶. The internal pressure of the medium being the same (~189 MPa) at a fixed NaCl concentration (0.2 M), the transition for Poly(dA).Poly(dT) would, therefore, require a higher temperature than that for Poly[d(A-T)], considering the basic thermodynamic relationship between pressure and volume with respect to the kinetics of two processes. Thus, T_m for Poly(dA).Poly(dT) is higher (73.1°C) than that for Poly[d(A-T)] (64.3°C). Thus, the value of ΔV , an indication of the hydration and other structural properties of any duplex, as described earlier, controls the variation in thermal stability at a definite internal pressure at a given NaCl concentration. Values of ΔV , thus, should be credited due importance in the transition reactions.

Pressure effects

Application of external pressure greatly influences the transition of duplex to single strands³⁶. In general, high pressure has been observed to result in an increase in T_m for several duplex-single strand transitions. The calculations in the present investigation of P_i for NaCl using high pressure data⁴⁵ at each applied external pressure value demonstrate that P_i increases with an increase in the external pressure. Thus, the total internal pressure of the system at a given concentration of NaCl is higher at an elevated external pressure than at atmospheric pressure. This increase in the total internal pressure, coupled with the positive volume of transition, inhibits the transition of duplex to single strands. In order to achieve the transition, therefore, a higher temperature is required when the external pressure is higher than the atmospheric pressure. For explaining our argument, we define two quantities: $T_m^R - 1$, (where T_m^R is the ratio of T_m at any external pressure to that at atmospheric pressure) and $P_i^R - 1$ (with an analogous definition as used for T_m). Some valuable information emerges out of plots

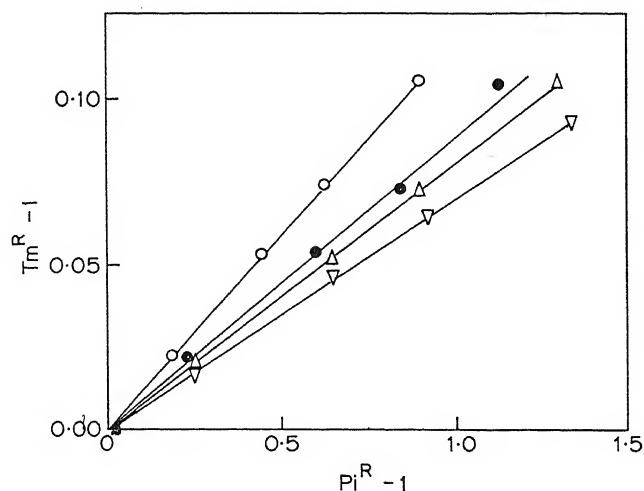


Figure 5. Relative transition temperature at various pressures. $T_m^R - 1$ as a function of relative internal pressures $P_i^R - 1$ at various pressures in several NaCl concentrations. O, 1 M; ●, 0.2 M; Δ, 500 mM; ▽, 200 mM duplex; Poly(dA).Poly(dT), range of external pressure covered 200 MPa; at atmospheric pressure (0.1 MPa) $T_m^R - 1 = P_i^R - 1 = 0$; points in plots are chosen to show extent of linearity, though linearity is observed throughout; experimental data from ref. 36; identical observations for other duplexes also.

(Figure 5) between $T_m^R - 1$ and $P_i^R - 1$ at several NaCl concentrations. For example, in the case of Poly(dA).Poly(dT), for which extensive data are available, the plots $T_m^R - 1$ versus $P_i^R - 1$ are linear at each NaCl concentration in the range from 200 mM to 1 M. The extent of linearity is seen up to 200 MPa with slopes of significant magnitude, suggesting the strong influence of internal pressure on thermal stability. It is, therefore, possible to characterize and correlate the thermal stability of DNA duplexes at several ionic concentrations under elevated external pressure conditions.

An important parameter, as pointed out in the foregoing discussion, is the magnitude and the value of ΔV . Two contributions of prime significance resulting into this property come from the base stacking and electrostatic effects. These effects give rise to negative and positive ΔV in the cases of formation of a duplex and transition of a duplex into two complementary strands, respectively. The base stacking effect originates from dispersion forces and hydrophobic effects, whereas the interactions between Na^+ and PO_4^- of DNA strands yield electrostatic effects. Though the signs of ΔV in the duplexation and the reverse reaction are negative and positive respectively, both positive and negative values are noted for duplexation in some cases. These very small positive values noted at extremely low Na^+ concentration are presumably the result of the combined effect of specific base pairing and the low degree of electrostriction. For example, Rentzeperis *et al.*²⁵ noted

positive ΔV at 16 mM Na^+ for Poly(rA).Poly(rU), which became negative at higher Na^+ concentration of 116 mM. Such variations in sign of ΔV have been described in terms of change in hydration of the DNA duplex as a function of ionic concentration.

Recalling the polyion condensation theory²⁷, thermodynamic binding of counter ion Na^+ with polyion PO_4^- is the major step. First, in presence of a salt, the counterion condenses on the polyion in order to reduce the axial charge density of a strand. This is followed by the second step, in which the remaining unneutralized polyions are shielded (screening effect) by an atmosphere of mobile counterions. It is not yet clear how much difference exists in counterion condensation in single strands and duplex²⁴. In the present approach, internal pressure induced by the salt is a bulk solution property and acts as a driving force for the formation of a duplex from its complementary single strands based upon the volume changes accompanying duplex formation. It appears that both the condensation and screening effects are indirectly accounted for in the internal pressure term of a salt solution. In our recent work, we have noted the magnitudes of both of these effects on the T_m values of the DNA duplexes, where the counterion condensation effects was noted to outcompete the screening effect. Details of these effects in terms of the pressure parameters are being communicated.

Conclusion and futuristic ideas

To summarize, the correlations exposed in the foregoing discussion are significant in many ways. First, the reaction rates and their spectacular enhancement in salt-solvent media can be quantified by a single concept based upon internal pressure and volume changes in reactions. This achievement which is still in the state of infancy needs rigorous testing. Secondly, a plausible explanation can be thought over for obviation of external ultrahigh pressure conditions during the synthesis of a compound. These two major achievements may eventually lead to the preparation of a selection software, which could be of potential use in recommending the reaction conditions to some extent. Thirdly, an alternate explanation is now available to describe the thermal stabilities of DNA duplexes at different ionic concentrations both at ambient and elevated pressures. Fourthly, since the analysis of thermal stabilities of DNA molecules is a radical departure from the presently-available explanations, there exists a new possibility to examine the physical etiology in sea water.

A brief account of the work discussed above, however, does not answer several important questions that a chemist or biochemist is faced with. An issue of both theoretical and industrial importance, which warrants urgent attention of the physico-organic chemists, is to

quantify the kinetic profiles of organic reactions of different types in terms of molecular interactions. Effect of additives, which are observed to alter the reaction rates and stereoselectivities of the products, is being currently investigated in detail.

How do the electrostatic forces or ion-water interactions govern the behaviour and biological functions of a single DNA strand and a triplex? How can we learn *a priori* about the activation parameters for duplex and triplex formation just from the knowledge of available information on the single DNA strands? A systematic study of duplexes made up of Peptide Nucleic Acid (PNA) and DNA as PNA-DNA for their thermal stability looks promising for designing of certain drug molecules following the concept of anti-sense technology. Calculations on these duplexes are yielding some exciting insights into the thermodynamics of nucleic acids, the results of which will be reported elsewhere.

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RESEARCH ARTICLE

Electrical resistivity imaging of Mohand Anticline of Siwalik range: A tectonic appraisal

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The results of a pilot resistivity sounding experiment undertaken in the Siwalik belt and contiguous Indo-Gangetic Foredeep (IGF) are presented and their lithotectonic significance is discussed akin to the Mohand anticlinal structure. The inferred large lateral resistivity contrast near Ganeshpur delineates the concealed Mohand thrust. The observed resistivity doublet is compatible with the transition in the clast-size and clast-matrix ratio of conglomerate

to sandstone. This correspondence provides strong geophysical support to the proposed four stage evolutionary model of the Mohand Siwalik basin on the basis of the facies association and palaeocurrent data. Cyclic pattern of resistivity distribution seen in the Mohand-Sherpur sector of IGF is considered to manifest episodic character of tectonic events in the Himalayan orogeny.

THE Indian Institute of Geomagnetism has been carrying out the natural source electromagnetic investigations in the Himalayas and the adjoining shield region¹⁻³. These studies, employing magnetovariational and magnetotelluric techniques, image the deep crustal structures in terms of electrical conductivity (inverse of resistivity) distribution. The strong dependence of conductivity on

temperature prevailing at deeper depths and the fluid content released and remobilized by the metamorphic and tectonic processes, makes these investigations a sensitive pointer of the geodynamic process controlling seismic and tectonic activity in the collision regime. These visualizations are well illustrated by the noted correlation of the mapped Trans-Himalayan and Garh-

wal Lesser Himalaya conductivity anomalies with the high seismicity zones^{4,5}. With the objective of supplementing information from shallow depths, it was decided to introduce control source deep electrical resistivity surveys. At shallow depths, the resistivity distribution being more sensitive to the state of saturation and/or to the fractured rock fabrics of the medium, the knowledge of resistivity distribution can effectively be used to infer the control of proximal or distal tectonics on the nature of sedimentation in the foredeep basins as well as in the alluvial fan system developed at the base of rising mountain system. We report in this paper the experimental details and results of a pilot resistivity survey undertaken in and around the Mohand structure of Siwalik range, Garhwal Sub-Himalaya.

Tectonic features of the study area and electrical sounding locations

For the resistivity sounding experiment, a section of the Siwalik range bounded between Yamuna and Ganga tear faults was selected. This section of the Siwalik fold belt lying on the southern flank of the Dehradun valley has received much attention from the Earth scientists for its anticlinal structure, Mohand anticline. The gentle NE dipping strata of the Siwalik range here show a sharp reversal of dip on its southern limit, giving the appearance of an anticline with its axis roughly aligned in NW-SE direction. The lithological sequence in the Siwalik range is represented by sandstone/mudstone couplets at the base, followed by multistoried sandstone complex and capped up by boulder conglomerate, respectively classified as Lower, Middle and Upper Siwalik⁶. Due to the anticlinal uplift, the Upper Siwalik in the crest region is extensively eroded, exposing the underlying Middle Siwalik on the SE flank. The Upper Siwalik are exposed on the NE flank abutting against the alluvium deposits of Dehradun valley. On the SE margin, the Siwalik range terminates into the Indo-Gangetic Foredeep (IGF). The contact boundary between the Siwalik range and alluvial deposits of the IGF, regionally called as Main Frontal Thrust or more simply as Himalayan Foothill Boundary (HFB)⁷, is locally referred to as Mohand Thrust. This thrust is inferred to be concealed under the alluvium deposit^{8,9}. The presence of the Mohand Thrust at depth was confirmed by borehole lithology encountered in structural and deep wells drilled by the Oil and Natural Gas Commission (ONGC) to evaluate hydrocarbon prospects of the Siwalik range⁸.

Four Schlumberger electrical soundings were conducted respectively at Kotri, Dharmawala, Ganeshpur and Sherpur (Figure 1) so as to provide maximum possible coverage of Mohand anticline. The first two sounding lines were located respectively on the southern and northern limb of the anticline. The Kotri sounding

line is located some 14 km northwest of Mohand whereas the Dharmawala sounding is on the northeastern flank of the anticline, close to the contact boundary of Upper Siwalik conglomerate sequence with alluvium. Both these sounding lines run parallel to the general NW-SE strike of the Mohand anticline. The Ganeshpur sounding line is located very close to the boundary between the alluvium and Siwalik with sounding line striking across the Mohand thrust. The fourth resistivity sounding line, centered near village Sherpur, was situated deep within the IGF, some 15 km south of Ganeshpur on the Dehradun-Saharanpur road.

The large variations in the range of apparent resistivities observed between Sherpur and Ganeshpur soundings warranted us to conduct axial dipole sounding to locate the lateral inhomogeneity between these locations.

Data acquisition and reduction

The Schlumberger resistivity sounding technique, adopted here, employs a co-linear electrode configuration symmetrically disposed to the central sounding point. A DC current I , in the form of square wave interrupted for the required time intervals through commutator switches, is injected into the ground by means of outer pair of electrodes A and B . The other pair of non-polarizable electrodes, M and N , placed close to the central point, is used to measure the potential difference, ΔV , resulting from the current flow. An apparent resistivity, ρ_a^{10-12} , is given by

$$\rho_a = \frac{\pi[(AB)^2 - (MN)^2] \cdot \frac{\Delta V}{I}}{4MN}$$

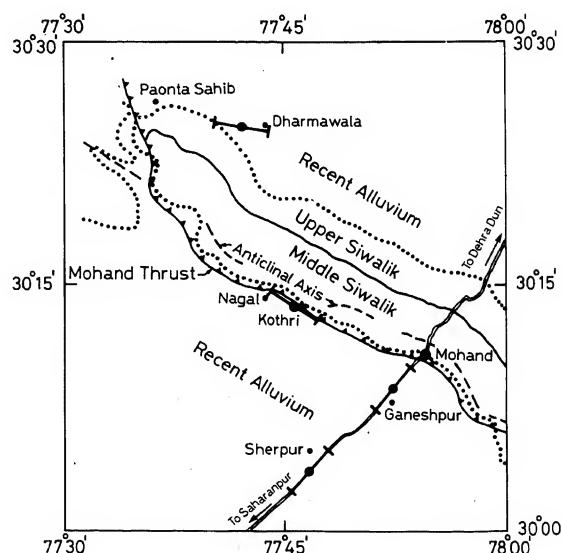


Figure 1. Map of study area showing the location of deep resistivity sounding lines on the Dehradun-Saharanpur road, undertaken to image electric resistivity distribution around the Mohand anticlinal structure.

The current electrode separation is increased systematically to achieve progressively deeper penetration. While moving apart the current electrodes in successive observations, the potential electrodes are kept fixed for a large number of the *AB* settings. The change in *MN* spacing is introduced only when the potential drop across the *MN* falls with increasing *AB* separation to a value close to the limiting precision of the receiver unit. This restricted repositioning of the potential electrodes helps to minimize the distortions resulting from the localized near-surface resistivity inhomogeneities. These effects, termed electrode effects¹³, could pose serious problem in the present case as the study region exposes a wide range of geological formations. The ratio of potential and current electrode separation was maintained between 1:5 and 1:10. This choice ensured adequate voltage drop across *MN* for the entire range of *AB*s.

The field measurements were accomplished with the Scintrex Resistivity Sounding equipment, essentially consisting of a current transmitting unit TSQ-4 backed up by 10 kW motor generator and a voltage measuring unit IPR-8. The motor generator generates three phase 210 Volts (AC) with a frequency of 600 Hz. This energy is transformed according to a front panel voltage setting in a large transformer housed in TSQ-4. The resulting AC is then rectified in a rectifier bridge. The commutator switch controls the DC voltage output in the square waveform interrupted at required time intervals. The IPR-8 receiver has a built-in facility to cancel self-potential existing at the potential electrodes. Driving the source current from generator and transmitter, maximum *AB* separation of 6 km was attained.

The apparent resistivity obtained as a function of electrode separation *AB/2* is plotted on the log-log scale to give usual representation of sounding curve. With a suitable choice of geometrical progression of current electrodes, a fairly uniform distribution of points on the sounding curve was obtained. The raw electrical sounding data as recorded at the Sherpur is shown in Figure 2. Often various segments of the sounding curve, each corresponding to a particular *MN* spacing, exhibit slight vertical shift in relation to adjacent branches. This invariably manifests the electrode effect associated with the repositioning of the *MN* electrodes, also due to the lateral inhomogeneities encountered between previous and present *MN* spacing. Since measurements with larger *MN* spacing involve larger volumes, they provide better average information. The shift in adjacent sections of the sounding curve is corrected by vertically shifting the segment of the sounding curve with smaller *MN* spacing so as to provide fit to the section of the sounding curve with larger *MN* spacing^{11,14}. Figure 2 demonstrates such a shift correction applied to the Sherpur sounding data. With these shift corrections, the overall shape of the sounding curve remains unaltered and only such smooth

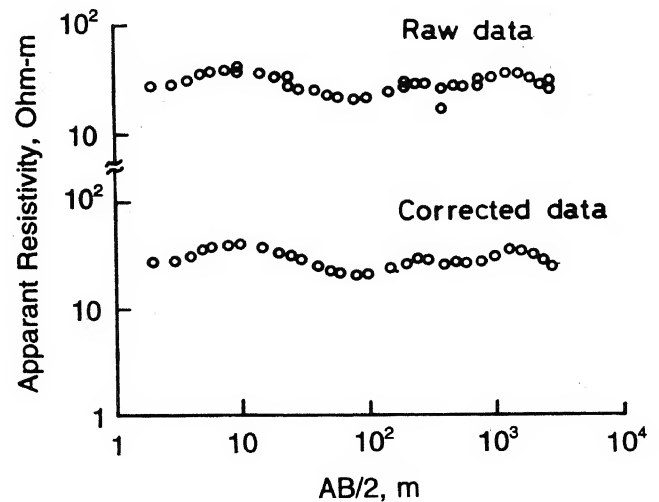


Figure 2. Plot of apparent resistivity vs electrode separation (*AB/2*) at Sherpur: as reduced from raw data (top) and after correcting (bottom) for electrode effect seen as vertically shifted segments of sounding curve.

sounding curves are used for subsequent interpretation of a 1-D layered structure.

For the two axial dipole soundings conducted, the current electrodes with 2 and 2.8 km separation were kept fixed while the potential electrodes with constant separation of 700 m were successively moved along the axis of current dipole with radial distances varying from 2 to 8 km on either side of the centre of the current dipole. The values of apparent resistivity for each position of potential dipole, calculated using the formula given below, were plotted as a function of radial distance of potential dipole from the central position of current electrodes to depict lateral variation of apparent resistivity along the line of movement.

The apparent resistivity is computed for the axial dipole configuration by the formula

$$\rho_a = \frac{\pi R^3}{AB \cdot MN} \cdot \frac{\Delta V}{I},$$

where *R* is the radial distance from the centre of the current dipole to the centre of potential dipole.

The axial sounding up to a radial distance of 8 km on either side of the Sherpur and Ganeshpur was undertaken with potential electrodes deployed approximately at every 2 km. Figure 3 shows the lateral variation obtained from two axial dipole soundings, the implications of which are discussed in the foregoing sections.

Numerical interpretation of sounding curves

The corrected apparent resistivity sounding curves have been interpreted by two different approaches, viz. the

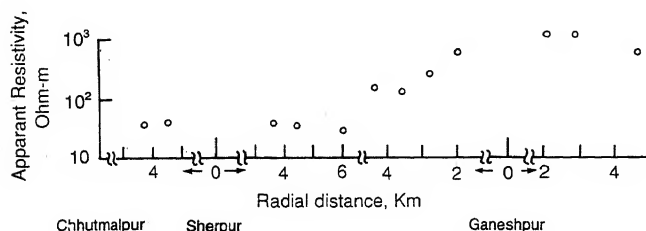


Figure 3. Plot showing the lateral variation in resistivity as obtained by axial-dipole soundings at Sherpur and Ganeshpur.

Marquardt-Levenburg¹⁵ and Occam's inversion¹⁶. The Marquardt-Levenburg (M-L) formulation interprets the resistivity data in terms of horizontal layer of isotropic resistivity. The method incorporating *a priori* information on the number of layers as well as initial approximations on layers parameters (thickness and resistivity), performs an iterative process to refine the model parameters while minimizing the sum of the squares of residuals between observed and calculated responses. The Occam's inversion, on the other hand, yields a smooth resistivity-depth model minimizing the integrated square of the first or second derivative of resistivity values with respect to the depth. This approach reduces the ambiguity in fixing up the number of layers in the layered-structured model of the Earth.

Figure 4 *a-d* shows the interpreted layered as well as smooth continuous resistivity-depth distribution models for all the four soundings conducted around the Mohand structure. The degree to which these models explain the data is illustrated in Figure 5 *a-d* by superposing the calculated response of the best fitting models, both layered as well as continuous, on the observed sounding data, separately for all profiles. The comparative study of these models revealed that the maximum and minimum in the Occam's model corresponded well with the resistivities of the layers seen as zones of relatively high and low resistivities. Further, the sharp resistivity discontinuities represented by layer boundaries match well with the inflection points between minima and maxima observed in the Occam's model. The correlation between the reduced resistivity models, obtained by the two techniques, provided confidence in the model parameters. Their primary features are summarized below:

Kotri and Dharmawala soundings

The Kotri and Dharmawala soundings, respectively on the southern and northern limb of the Mohand anticline, present a nearly consistent picture. The Kotri sounding with a thin overburden of modest resistivity (70 ohm.m) indicates a high resistivity layer of about 550 ohm.m

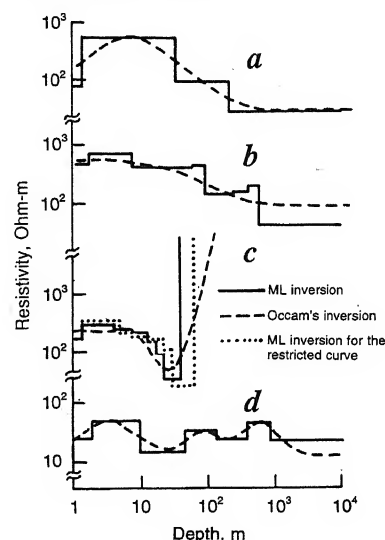


Fig. 4

Figure 4 *a-d*. Interpreted layered (solid line) as well as continuous resistivity-depth distribution (broken-line) models for resistivity sounding curves at (a) Kotri, (b) Dharmawala, (c) Ganeshpur and (d) Sherpur. For Ganeshpur, results are based on the interpretation of restricted (dotted line) and full (broken) sounding curve (see text).

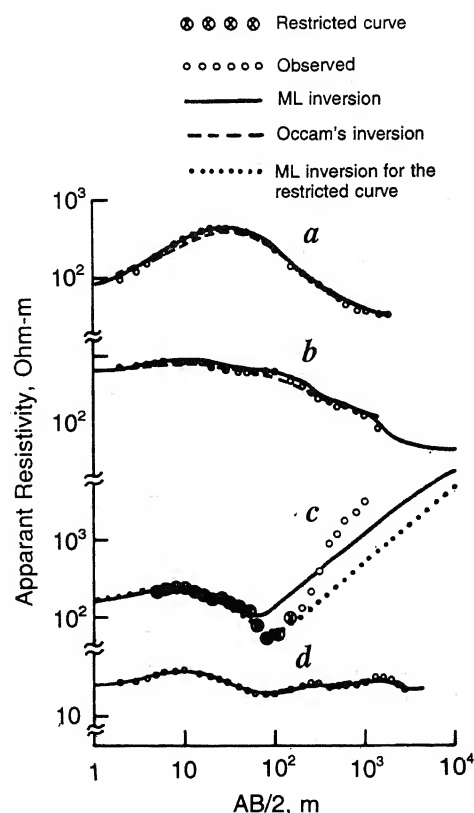


Figure 5 *a-d*. Plots showing calculated responses of best fitting layered (solid) and continuous depth-resistivity (broken line) models, shown in Figure 4, to the observed data (hollow circles) at (a) Kotri, (b) Dharmawala, (c) Ganeshpur and (d) Sherpur. For Ganeshpur and Sherpur, the Occam's and ML, inversion fits are overlapping and hence the broken line cannot be shown separately. The dotted line represents ML, fit for the restricted part of the curve indicated by crossed circles.

having an approximate thickness of 30 m (Figure 4a). The interpretation of the Dharmawala sounding indicates near-equivalent resistivity (ranging between 425 and 740 ohm.m) layer at the surface, extending up to the depth of about 90 m (Figure 4b). On both the soundings, this high resistivity layer is underlain by a relatively less resistive layer. The 4th layer on the Kotri profile has resistivity of nearly 90 ohm.m. This layer is not resolved by the Dharmawala sounding. Of the four soundings operated, the Dharmawala sounding is most strongly affected by the electrode effects. This, coupled with high contact resistance at the current electrodes, has greatly reduced the depth penetration of current. The non-detection of the moderately resistive layer at Dharmawala may be the effect of this restricted penetration, although the presence of such a layer cannot be ruled out by the continuously decreasing trend of the sounding curve at large electrode separation.

Sherpur sounding

In marked contrast to the soundings located on the Mohand anticline, the Sherpur sounding profile located only 15 km south of the HFB into the Indo-Gangetic foredeep, is characterized by low resistivities. Here the resistivity-depth distribution, in the upper 1000 m section, is represented by an 8-layered structure (Figure 4d). The resistivity of the individual layers ranges between a narrow range of 17 and 50 ohm.m, a distinct feature being that resistivity distribution alternates between relatively low and high resistivity layers. The layer of lowest resistivity (17 ohm.m) extending from 10 m depth, with a thickness of approximately 40 m, appears to represent the aquifer. The depth of this layer is in good agreement with the depth of the water table as revealed by the existing wells in the vicinity of the sounding.

Ganeshpur sounding

The Ganeshpur sounding, placed close to the HFB, exhibits an H-type curve (Figure 5c), symbolizing the presence of a low resistive layer sandwiched between resistive layers. However, the sounding curve when the electrode separation AB exceeds 100 m, rises very steeply. Both the Occam and $M-L$ inversion schemes fail to yield a single model providing match for the complete sounding curve (Figure 5c), especially the steeply rising segment. The last segment of the sounding curve will be asymptotic to a line rising at an angle 45° when the lowermost layer is an insulator^{10,12}. However, the observed slope of the experimental sounding curve at Ganeshpur is more than 45° which cannot be explained even by the insulated bottom layer. Hence, this may be visualized as a diagnostic of the presence of

large lateral electrical resistivity contrast at shallow depth along the sounding line. The interpretation of the Ganeshpur sounding curve up to an electrode separation of 100 m by the $M-L$ algorithm, indicated that this section of the sounding curve is compatible with a top resistive layer of 200–300 ohm.m underlain by a low-resistivity layer of 20 ohm.m with an approximate thickness of 33 m extending from the 30 m depth. Assigning these values to a 3-layer model, with only the resistivity of the infinite third layer as a free parameter, the $M-L$ algorithm was made to search by iterative process the resistivity which could provide the closest fit to the rising part of the Sherpur sounding curve. It was found that even when the bottom layer attains a value of the order of 20,000–25,000 ohm.m, the overall fit is not satisfactory and also the match to the first section of the sounding curve is seriously distorted (see Figure 5c). This exercise demonstrated that even a near-insulating substratum at a shallow depth of 60–70 m is not consistent with observations, providing further credence to the hypothesis of large lateral resistivity inhomogeneity. It can be surmised that as the current with increasing current electrode separations tends to penetrate to the depth of the discontinuity, the capacitor-like behaviour of the electrical inhomogeneity tends to drain away the electrical energy (R. J. Sporry, personal communication), resulting in the steep rise of sounding curve after certain threshold of the electrode separation.

It is noteworthy that estimated depth of low resistivity zone (20 ohm.m), obtained by modelling the curtailed sounding curve, agrees well with the water level in a single well located only a few meters away from the sounding centre. Comparison with Sherpur model suggests that aquifer located at 10 m depth deepens to a depth of 30 m near Ganeshpur.

Axial dipole sounding

The results of the axial soundings provide independent confirmation of the lateral resistivity inhomogeneity, a little north of Ganeshpur. The examination of lateral variation plot of resistivity in Figure 3, as estimated from the axial dipole sounding centered at the Ganeshpur, indicates that apparent resistivity on the north-east part is more than 1000 ohm.m as compared to the apparent resistivity of 200–300 ohm.m on the SW part. The axial dipole sounding results centered at the Sherpur revealed uniform value between 30 and 40 ohm.m on either side. These results indicate that near-homogeneous resistivity distribution in the IGF tends to be progressively inhomogeneous as the HFB is reached, with HFB marking zone of sharp resistivity contrast. The deduced lateral resistivity distribution shows good semblance with the exposed lithology of the area. The alluvium-covered region of the IGF is marked by low

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and laterally uniform resistivity whereas the transitional and high-resistivity zones respectively match the pebble mixed alluvium and boulder sequence of the region.

Geological and tectonic bearings

The results of the present resistivity sounding experiment indicate that Mohand Thrust, marked by large lateral resistivity contrast, is located in close proximity to the centre of Ganeshpur sounding. The thrust is indicated to be concealed approximately at a depth of about 40–50 m. Due to the known sensitivity of control source electrical method to resolve resistive structure⁴, the electrical resistivity soundings elsewhere on the Mohand anticline have been able to divide upper pile of sedimentary sequence into a multiple layer sequence. Figure 6 summarizes the results of resistivity soundings in the form a geological cross-section corresponding to shallow depths. The picture of deeper section is adapted from Raiverman *et al.*⁷.

In agreement with the exposed lithology, the top resistive layer (~550 ohm.m) at Dharmawala is shown to correspond with the boulder-bearing sequence of the Upper Siwalik. Extending the analogy, the second resistive layer at Kotri with similar resistivity character is also identified in Figure 6 as boulder dominated conglomerate sequence. Both at Dharmawala and Kotri, this layer is underlain by a relatively lower resistivity

(200 ohm.m) layer. Making in-depth examination of the stratigraphic sections in and around Mohand Siwalik, Kumar and Ghosh⁶ identified four distinct facies association occurring vertically upward as A (sandstone-mudstone), B (sandstone), C (sandstone-conglomerate) and D (conglomerate) the facies association C and D occurring mostly in the Upper Siwalik.

They observed that facies association C is characterized by stratified conglomerate with clast-ratio determined by 30–50% of conglomerate and 40–60% of sandstone. The facies association C gradually passes upward into poorly-imbricated conglomerate sequence in which conglomerate:sandstone ratio changes to 70:30. The clast size also varies from 10–25 cm in facies association C to 25–40 cm in association D. It is interesting to note that resistivity changes observed between resistive doublet at Dharmawala and Kotri are compatible with this transition in the clast-size and the clast-matrix ratio, the upper resistive and underlying relatively less resistive layers respectively representing formations dominated by facies D and C.

Clearly, the ability of the control source electrical method to sub-divide the Upper Siwalik sequence into two distinct layers, arising due to lithological change, can be considered as geophysical support to the four-stage evolution model of Mohand Siwalik basin, proposed by Kumar and Ghosh⁶, with two stages of sedimentation occurring during Upper Siwalik.

At Kotri, this resistive doublet is sandwiched between moderately resistive layers of 70–150 ohm.m. These resistivities are typical of sandstone¹⁷ and are interpreted as representing the sandstone sequence of Middle Siwalik. While the lower layer may mark the Middle Siwalik sequence brought close to surface by compressional forces responsible for anticlinal structure, the top moderately-resistive layer is considered to denote the debris derived from the erosion of Middle Siwalik exposed near the crest of anticlinal structure. Further south, near Ganeshpur, the contact between the concealed resistive Upper Siwalik and alluvium deposit may provide the kind of lateral resistivity contrast mapped by Ganeshpur sounding and axial dipole resistivity surveys. Compressional stresses associated with anticlinal folding may also cause weak flexing of the basement in the immediate vicinity of the southern limb of the anticline and may account for the deepening of the water-bearing sedimentary column as compared to the Sherpur. The subsequent filling of this basin-like structure with the boulders brought down by rising Mohand anticline may provide surface cover, seen here as a surfacial resistive (200 ohm.m) layer near Ganeshpur, concealing the contact between Siwalik and alluvium. Intermixing with the channelized or braided river deposits may lower the resistivity of this boulder sequence as compared to the Upper Siwalik boulder conglomerates seen on the limbs of the anticline.

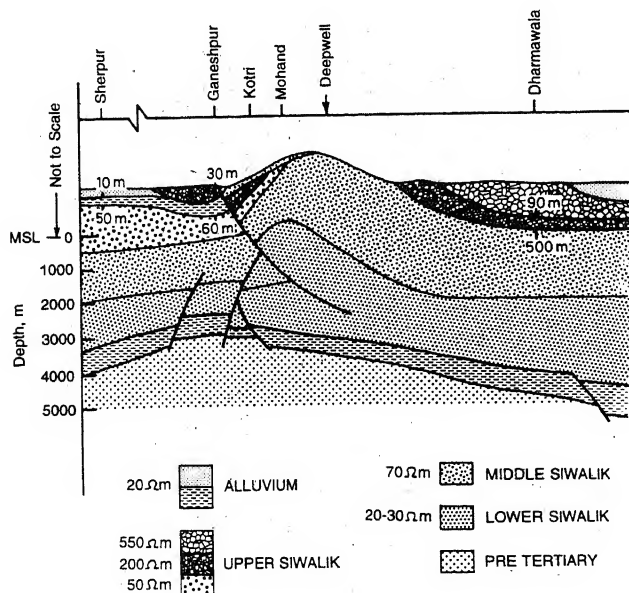


Figure 6. Schematic geological cross-section across the Mohand anticlinal structure, incorporating features of electrical resistivity distribution estimated from the present study. The vertical scale for shallow section is highly exaggerated but depths of mapped interfaces at each of the sounding sites are shown. The deeper section is adapted from Raiverman *et al.*⁷.

The most interesting feature of the resistivity–depth distribution at Sherpur is the repetition of high and low resistivity couplet. Since the development and nature of sedimentation in the foredeep basin is largely caused and constrained by the tectonic history of the orogen, it is tempting to relate their cyclic distribution of resistivity to the episodic tectonism in the evolution of the Himalaya. The high resistivity layer may be visualized as coarse-grained sedimentary formation due to the rapid tectonic impulses. Superimposed on this is the slow sedimentation during a long quiescent period. The slow sedimentation results in fine-grained rock formations symbolizing the low resistive layers. Further, Bhattacharya and Misra¹⁸ conclude that during most of the middle Siwalik times, the sands were being deposited by streams due to the floods at repeated intervals. They have also visualized that the Upper Siwaliks were deposited under the proglacial conditions in the Mohand area by streams originating from melt waters at the ice front of mountain glaciers. Sinha⁹ pointed out that the sediments of the Siwalik of Mohand were derived from some mobile belt occurring towards east and northeast and consisted of igneous and metamorphic rocks of the Himalayan belt. This mobility, a series of episodic tectonic events, is a manifestation of the general uplift of the Himalaya during the post-Oligocene periods¹⁹.

The relatively low resistivities of all the layers in the IGF as compared to the Siwaliks may be due to the moist conditions prevailing in the foredeep. However, the alternative resistivity highs and lows can be effectively correlated to the episodic tectonic events in the Himalayan belt, if a comprehensive study on the integrated geological and geophysical data is attempted in this sector of Indo-Gangetic Foredeep.

Conclusions

A pilot electrical resistivity survey in and around the Mohand structure has unambiguously located the Mohand thrust. Elsewhere the mapped resistivity distribution is compatible with the lithology and has been helpful in constraining the geological cross-section of the Mohand structure at shallow depths.

The top Upper Siwalik sequence on the NE flank is seen as a resistive doublet. Similar doublet embedded between relatively less resistive layers is identified on the SW flank.

This might warrant that during the anticlinal upliftment, Upper Siwalik sequence may not have been com-

pletely eroded on the SW flank. The exposed sandstone sequence on this flank may simply be the debris of eroded Middle Siwalik from the crest region beneath which lies the normal Upper and Middle Siwalik sequence.

The cyclic distribution of resistivity at Sherpur may be visualized as manifestation of the episodic tectonism.

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Interstratified low-Ti and high-Ti volcanics in arc-related Khairagarh Group of Central India

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We report here the petrotextonic implications of interstratified low-Ti and high-Ti volcanics in the Khairagarh metabasalts of Sitagota Syncline, Central India. The low-Ti volcanics have higher CaO/TiO_2 , $\text{Al}_2\text{O}_3/\text{TiO}_2$ ratios and LILE (?), LREE, Zr, Hf, and Cr concentration and distinct negative Eu anomalies, and lower FeO^T/MgO , P, V, Sc, HREE than high-Ti volcanics. Although low-Ti and high-Ti volcanics are both arc-related tholeiites, they are not related to each other by crystal fractionation and/or crustal contamination processes. They represent different degrees of partial melting of heterogeneous mantle source under different subduction fluxes, $\text{P}_{\text{H}_2\text{O}}$ and fO_2 conditions.

INTERSTRATIFIED low-Ti and high-Ti volcanics are a characteristic feature of arc-related volcanics globally¹⁻⁴. They have also been reported from many ophiolites⁵. Recently, several geochemical studies have suggested that the younger Khairagarh volcanics of the Dongargarh Supergroup have an arc-related tholeiitic affinity⁶⁻⁸. We report here for the first time, the occurrence of two chemically coherent geochemical groups of interstratified volcanics from the Early Proterozoic (~2.1 Ga) (ref. 23) Sitagota and Mangikhuta Metabasalts of Central India: one higher in TiO_2 (> 0.7 Wt.%) and the other, subtly but distinctly lower in TiO_2

(< 0.7 Wt.%). We suggest the occurrence of similar low-Ti and high-Ti volcanics from other parts of the Khairagarh volcanics, albeit with different minor elemental geochemical characters^{7,9,10} (Figure 1). These observations have important petrotextonic implications for the role of 'subduction-related processes' in the evolution of the Central Indian craton in particular, and the nature of Early Proterozoic global tectonics, in general.

The low-Ti and high-Ti volcanics reported in this communication are fine- to medium-grained metabasalts. Overall, they have retained their relict textures that includes subvolcanitic, subophitic to ophitic and porphyritic types. However, except for relict clinopyroxenes, all other magmatic phases have been replaced by secondary mineral assemblages during alteration(s) and prehnite-pumpellyite grades of metamorphism. Petrography reveals that olivine (?) was followed by clinopyroxene and plagioclase as fractionating phases. The magmatic clinopyroxenes are augites, in both low-Ti and high-Ti volcanics and paradoxically have overall similar chemistry, including their overall Ti contents but with different Ti/Al ratios.

Representative analyses of primitive low-Ti and high-Ti volcanics are given in Table 1. Samples were analysed at NGRI, Hyderabad by XRF and ICP-MS. Analytical techniques, accuracy and precision of the analyses are given elsewhere^{11,12}. The low-Ti and high-Ti volcanics are rather primitive by island-arc standards, in terms of high MgO, Cr and low Al_2O_3 contents. The basaltic (SiO_2 < 52 wt.%) to basaltic andesites (SiO_2 : 52 to 55 wt.%) of the low-Ti volcanics have high MgO (~12 to 7 wt.%) and Cr (~850 to 250 ppm), whereas high-Ti basalts (excluding an anomalous basaltic andesite) have MgO between 8.4 wt.% and 7.2 wt.% and Cr between ~350 ppm and 85 ppm. The high-Ti volcanics have higher concentrations of FeO^T (~17 wt.% to 15 wt.%) compared to the low-Ti volcanics (~14 wt.% to 12 wt.%). The high-Ti volcanics are more evolved in terms of their FeO^T/MgO ratios, whereas in terms of SiO_2 , the low-Ti volcanics are more evolved. In the (Zr + Y) vs ($\text{TiO}_2 \times 100$) vs Cr plot¹³, the low-Ti suites are classified as magnesian tholeiites, whereas the high-Ti suites are normal tholeiites. In the CaO/TiO_2 and $\text{Al}_2\text{O}_3/\text{TiO}_2$ versus TiO_2 plots (Figure 2), the low-Ti suites of all the Khairagarh volcanics have higher CaO/TiO_2 and $\text{Al}_2\text{O}_3/\text{TiO}_2$ ratios and spread around the primitive (i.e. modal pyrolite) compositions, whereas the high-Ti suites have lower CaO/TiO_2 and $\text{Al}_2\text{O}_3/\text{TiO}_2$ ratios, and plot away from the primitive composition. Assuming a simple pyrolite source, this diagram indicates that the low-Ti and high-Ti volcanics represent higher and lower degrees of partial melting, respectively⁵.

The low-Ti volcanics show LREE enriched patterns (Ce/Sm_N : 2.43 and 2.85), whereas the high-Ti volcanics show moderately enriched LREE patterns (Ce/Sm_N : 1.55

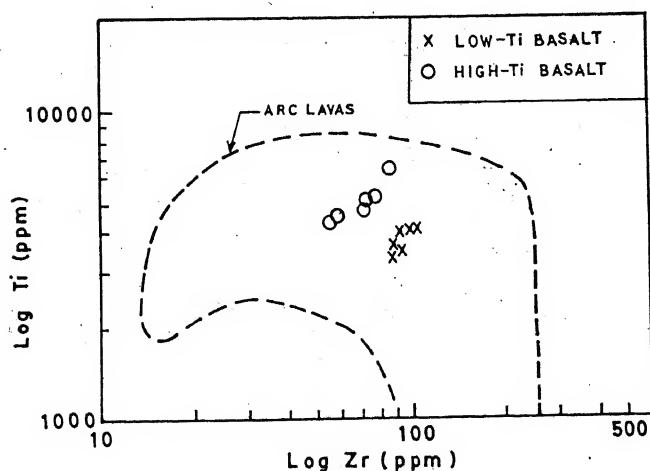


Figure 1. TiO_2 vs Zr plot showing two distinct trends in Khairagarh Volcanics, Sitagota Syncline. Also shown is the volcanic-arc lava field of Pearce (1982).

Table 1. Representative analyses of low-Ti and high-Ti Sitagota Metabasalts, Central India

Elements	Sample no. 34	Sample no. 13
	(wt. %)	
SiO ₂	50.75	49.50
TiO ₂	0.53	0.93
Al ₂ O ₃	13.50	14.65
FeO(T)	14.17	15.17
MnO	0.19	0.23
MgO	11.97	8.36
CaO	7.08	8.50
Na ₂ O	1.01	2.57
K ₂ O	0.69	0.52
P ₂ O ₅	0.10	0.10
LOI	1.52	7.10
Total	100.85	100.75
	(in ppm)	
Sr	130	232
Rb	35	17
Ba	352	225
Th	6.9	1.4
U	2.7	0.4
Nb	1.7	—
Zr	94	57
HF	2.5	1.8
Y	21	26
Sc	26	41
Cr	841	345
Ce	32	16
Pr	4	2.2
Nd	15	9
Sm	3	2.6
Eu	0.8	0.9
Gd	3	3
Dy	2.9	3.6
Er	2.0	2.3
Yb	1.6	2.4
Lu	0.2	0.4

to 1.85). The HREE patterns of the low-Ti volcanics are almost flat (Gd/Yb_N : 1.29 to 1.67), whereas the high-Ti volcanics have flat to moderately fractionated HREE patterns (Gd/Yb_N : 1 to 2.01). The contrasting REE patterns together with subtle major and minor element differences rule out the possibility of relating the high-Ti and low-Ti volcanics by any conceivable fractionation processes¹⁴. The primitive nature of these samples rule out the possibility of crustal contamination to relate them¹⁴⁻¹⁶.

Another distinct feature is the presence of pronounced negative Eu anomalies in the low-Ti volcanics, indicating plagioclase fractionation. In island-arc related suites, early plagioclase removal takes place under low P_{H_2O} conditions^{17,18}. This is also corroborated by the behaviour of V, which increases with fractionation in the low-Ti volcanics, whereas lack of plagioclase removal in the high-Ti volcanics (i.e. no Eu anomaly) indicates relatively high P_{H_2O} condition, however, not high

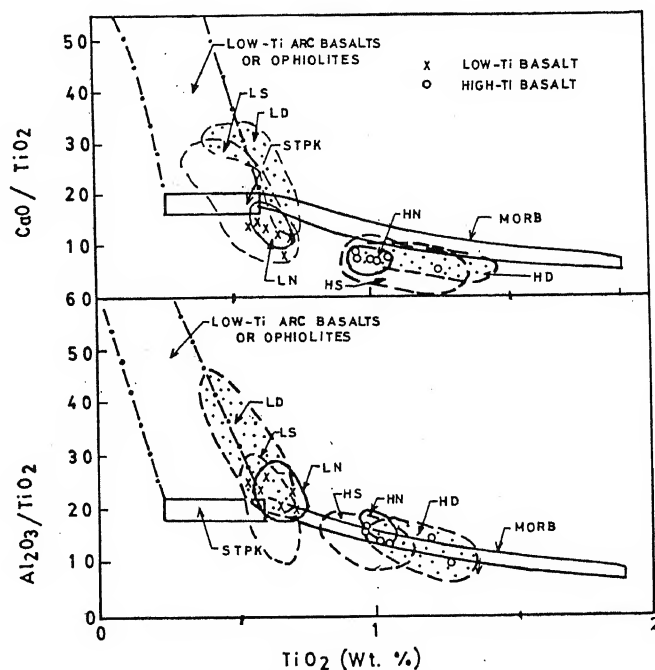


Figure 2. TiO₂ vs CaO/TiO₂ and Al₂O₃/TiO₂ plots (after Sun and Nesbitt⁶) for high-Ti and low-Ti volcanics of Khairagarh Group. Fields of Mid-oceanic Ridge Basalt (MORB), Spinifex-textured periodotitic komatiites (STPK) and low-Ti arc basalts or ophiolites⁶. Fields of low-Ti volcanics (LS, LD and LN respectively) and high-Ti volcanics (HS, HD and HN respectively) are for Khairagarh volcanics^{7,9,10}.

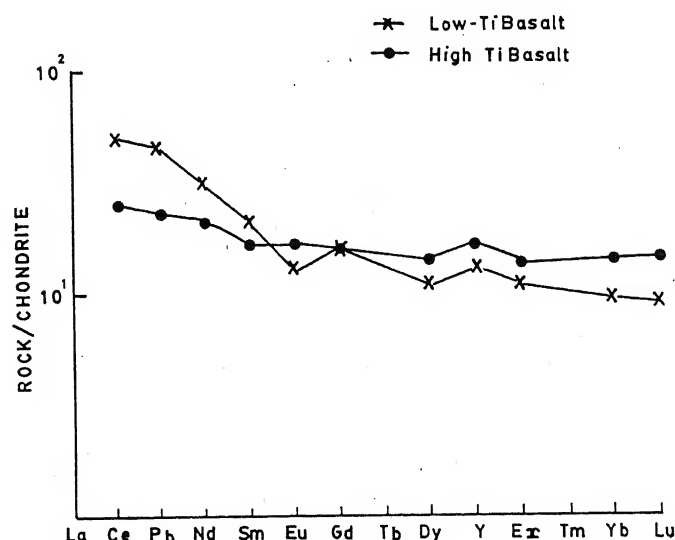


Fig. 3

Figure 3. Chondrite normalized REE patterns of representative samples of low-Ti and high-Ti volcanics, Khairagarh Group.

enough to produce calc-alkaline volcanics^{17,18}. High P_{H_2O} conditions in the generation of high-Ti suite is corroborated by the falling concentrations of V with fractionation. V versus Yb plot¹⁹ also indicates relatively

higher f_{O_2} conditions in the high-Ti suite, compared to the low-Ti volcanics; between QFM and higher than QFM + 1.

The mantle source of the low-Ti volcanics was LREE enriched (i.e. E-MORB and/or higher subduction flux²⁰) compared to the high-Ti volcanics. Higher proportions of garnet in the mantle residue of the low-Ti volcanics retained Ti, HREE and Sc compared to the high-Ti volcanics. Therefore, the subtle major and minor element differences between the arc-related high-Ti and low-Ti volcanics (Figures 1–3) are neither related to elemental mobility, nor can they be explained by clinopyroxene and plagioclase fractionation²¹, but are due to different degrees of partial melting of a heterogeneous mantle source (with respect to garnet) under different P_{H_2O} , f_{O_2} and subduction fluxing conditions.

The enriched MORB source of the low-Ti volcanics implies early stages of back-arc spreading, as in the Izu-Marianas arc or interarc rifting²⁰ and provides a simple explanation for the paradoxical presence of interlayered sedimentary formations of the Khairagarh Group⁷. Similarly, the presence of only tholeiitic lavas in the Khairagarh volcanics indicates the subduction of a relatively young and thin (< 20 km) oceanic plate with relatively high (> 7 cm/yr) convergence rates²².

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Unusual aspects of pressure-induced phase transitions in CuGeO_3

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High pressure Raman and optical studies on the spin-Peierls compound CuGeO_3 have revealed three novel pressure-induced phase transitions in the 7–8 GPa range. One of these occurring only under hydrostatic conditions, is believed to be a ferroelastic-ferroelectric phase. The other two high pressure phases are brilliant blue and green, respectively. Of these, the blue-phase is quenchable, while the green-phase reverts to the blue. From crystal chemistry, Raman spectral features and metastability considerations, it is suggested that the blue and the green phases have the pyroxene type chain structure similar to enstatite polymorphs, and non-hydrostatic pressure is essential for the formation of pyroxene type chain structures.

COPPER metagermanate (CuGeO_3) is a very interesting material, for it is the first inorganic system that exhibits the so-called spin-Peierls transition¹, when cooled below 14 K at ambient pressure. It is a very soft layer-type light blue material (like mica), crystallizing in the orthorhombic space² group Pbmm (C_{2h}^{52}). The backbone of the structure consists of chains (parallel to the c -axis of the crystal) of corner shared GeO_4 tetrahedra linked by edge-sharing CuO_6 octahedral chain co-parallel to the GeO_4 chain², as shown in Figure 1 on the left. The structure is related to the pyroxene family minerals, viz. enstatite, but differs from it in having a unique arrangement of GeO_4 tetrahedra called 'einer' chain, stabilized

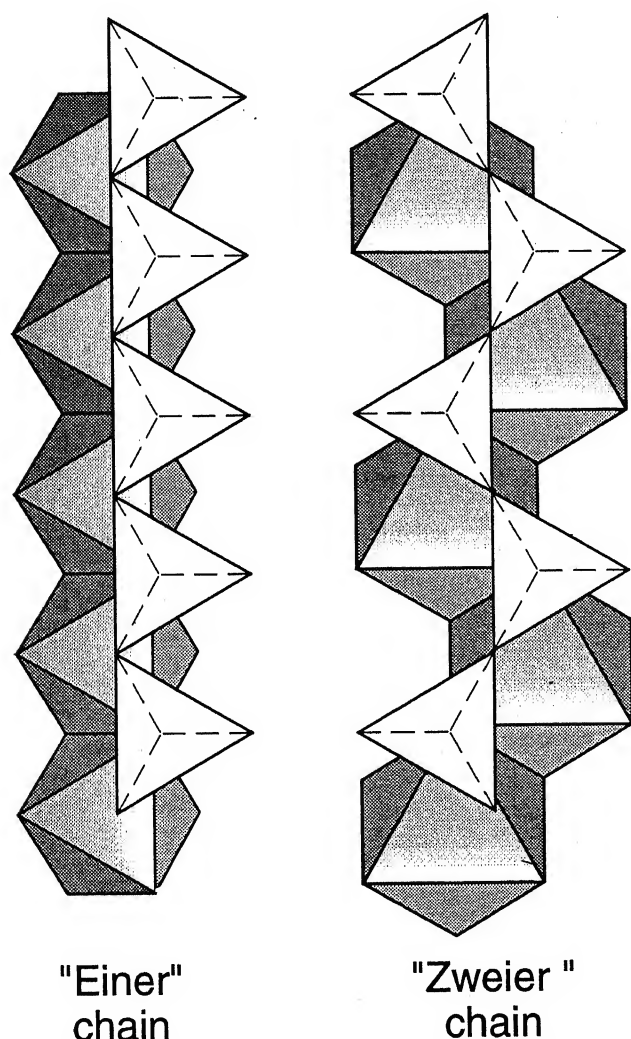


Figure 1. The tetrahedral GeO_4 corner sharing extended 'einer' chain with the CuO_6 edge sharing octahedral chain, the backbone of the CuGeO_3 structure. On the right is a pyroxene type extended 'zweier' chain. The chains run parallel to the c -axis of the crystal. Note that all the tetrahedra are on the same side in 'einer' chain whereas in the pyroxene type chain they alternate between the two sides of the chain.

by the tendency of the copper to have Jahn–Teller distorted CuO_6 octahedra. In the 'einer' chain, all of the tetrahedra units lie on the same side of the chain, while in a pyroxene chain they lie on either side (see Figure 1 right side).

From the point of view of high pressure behaviour also, CuGeO_3 is turning out to be very interesting. Our previous high pressure Raman and optical studies on the system³ have revealed the occurrence of a rather unusual type of reversible phase transition near 7 GPa, when 4:1 methanol/ethanol mixture was used. Further extensive studies of the high pressure behaviour of CuGeO_3 carried out recently using Raman spectroscopy and optical microscopy, with a variety of pressure media and with

single crystal and powder samples have yielded a wealth of details. The high pressure behaviour of CuGeO_3 is spectacular and quite complex. We will illustrate some novel aspects of the pressure-induced transitions and discuss them briefly here. A detailed account will be published elsewhere.

Three distinct high pressure phases are encountered in the 7–9 GPa region, when the normal orthorhombic (Phase I) Pbmm (D_{2h}^3) phase of CuGeO_3 (Pmma in the standard notation) is compressed. Surprisingly, the type of phase obtained depends on the hydrostaticity of the pressure medium near 8 GPa. This is shown diagrammatically in Figure 2. Thus when methanol/ethanol mixture, pure methanol or helium is used, the sample abruptly contracts by about 15% in the b -direction of the crystal, near 7 GPa. The resultant high pressure phase which we label as Phase II is pale green and has a different Raman spectrum. On pressure release, the reverse transition takes place near 5.6 GPa. This phase (I to II) transition was first observed in methanol/ethanol mixture earlier, and its amorphization above 15 GPa was documented in the earlier publication by Jayaraman *et al.*³ In the present study, we have observed this very same transition with helium which is a perfect hydrostatic medium up to 11.8 GPa at room temperature⁴, and also in pure methanol which freezes just a little above 7 GPa. The photomicrographs reproduced in Figure 3 show a single crystal sample of CuGeO_3 at different pressures in helium gas as pressure medium in the diamond cell. Figure 3c is the high pressure phase (Phase II). The contraction in the b -axis direction is striking. In Figure 4, the Raman spectrum of CuGeO_3 at 1 bar and at 7.9 GPa in helium is presented.

When argon is used as a pressure medium, the original phase starts to turn blue (designated Phase III) near 7.5 GPa. While this transition is progressing, another phase transition intrudes and the colour of the sample changes to green (designated Phase IV). This transition starts as green stripes traversing the crystal, and then covers the entire sample with increasing pressure. The change in the colour is accompanied by changes in the Raman spectral features. The green phase is stable up to 15 GPa, as judged from its Raman spectral features. When pressure is released, the green-phase transforms to the blue phase near 5 GPa and the latter can be quenched to ambient pressure. The quenched blue phase can be reversed to the original orthorhombic phase, only by heating to 600°C. The blue phase on repressurization transforms to the green phase near 8 GPa and the transformation can be recycled back and forth, irrespective of the pressure medium used. Further, there are no striking changes in the sample dimension accompanying the phase III \rightleftharpoons IV transitions, and the single crystal nature is preserved. In Figure 5, colour photomicrographs of Phase I at two pressures and Phase III and IV at 4 GPa (pressure release cycle) and 11 GPa respectively are

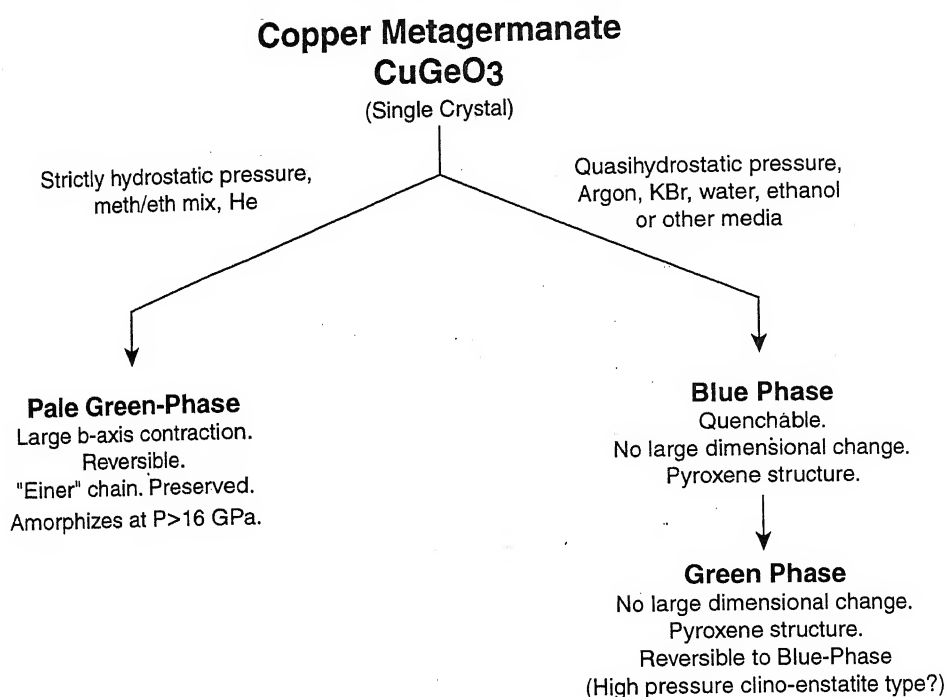


Figure 2. Diagrammatic representation showing the forking of the pressure-induced phase transitions in CuGeO₃, depending on the nature of stress.

presented. The pressure medium was argon. The Raman spectra of the green phase (IV) recorded near 5.6 GPa, and of the blue phase (III) near 4 GPa (both on the pressure release cycle) are shown in Figure 6.

With all other pressure media, the results obtained are similar to those described in the case of argon since they freeze before 7 GPa. Argon freezes at 1.2 GPa at room temperature. Potassium bromide (KBr) is a soft solid and therefore, behaves as a quasihydrostatic medium.

The results presented raise two main questions: (1) what are the structures of the three high pressure phases and (2) why does the hydrostaticity of the pressure medium have such a strong influence on the nature of the phase transition? Obviously a definite answer to the first question must come from high pressure X-ray diffraction studies on the high pressure phases, preferably single crystal diffraction. An earlier energy dispersive X-ray diffraction study⁵ on CuGeO₃ powder up to 20 GPa reported a phase transition near 7 GPa. For the high pressure phase, a monoclinic cell of $P2_1/m$ symmetry has been proposed. Quite recently, Haines and Adams⁶ have commented that the phase transition reported by us on single crystal CuGeO₃ (ref. 3) must be the same as the orthorhombic to the monoclinic phase mentioned, and not due to intercalation, as we had suggested. Our experience with CuGeO₃ powder is that the Phase I to II transition does not occur unless pressurization is carried out in a hydrostatic medium. From our new findings presented in this paper, it appears to us that the phase transition reported in the X-ray study is most

likely to be from the normal orthorhombic to the green phase.

We will now discuss some possible structures for the blue and the green phases of CuGeO₃, in the light of the known high pressure crystal chemistry of silicate pyroxenes^{7,8}, and the structural features of other metagermanates⁹. In silicate pyroxenes, the SiO₄ tetrahedra form infinite chains by corner sharing along the *c*-axis of the crystal, and the chains are linked by cation polyhedra to form the structure. The tetrahedra lie on either side of the chain (see Figure 1) connected by bridging oxygens and is called a 'zweier' chain, because the periodicity along the *c*-axis is twice the distance of the bridging oxygens. The most important difference between the pyroxene and CuGeO₃ is the so-called 'einer' chain feature, which is unique to this compound². The stability of this chain is believed to be very low because of the strong repulsion of the highly-charged Ge⁴⁺ ions¹⁰. It is the energy gain resulting from the Jahn-Teller distortion of Cu that stabilizes the structure. The pyroxene type 'zweier' chain appears more natural for CuGeO₃. In fact MgGeO₃ has the usual pyroxene type GeO₄ chain and is known to crystallize in the ortho-enstatite (Pbca) and clino-enstatite (C2/c) structure⁹. Thus, we can expect CuGeO₃ to transform from the 'einer' chain type to the pyroxene type of structure at high pressure.

For the blue phase, we suggest the ortho (Pbca) or the low clino-enstatite structure (C2/c), based on crystal chemistry, Raman spectral feature and quenchability. Thus, (i) the magnesium compound MgGeO₃ under

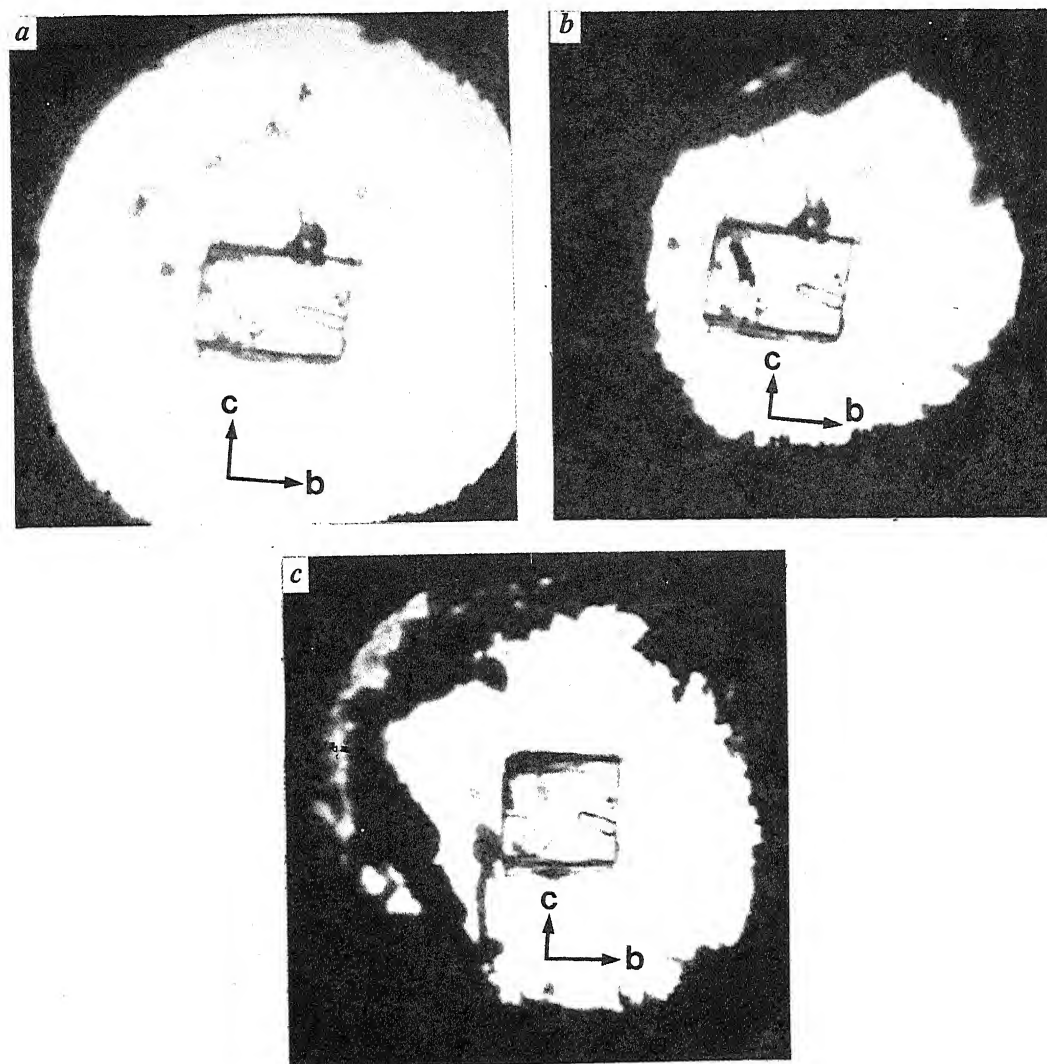


Figure 3 a–c. Photomicrographs of CuGeO_3 single crystal in the diamond cell at three different pressures, with helium as pressure medium (a) 0.5 GPa (b) 5.2 GPa and (c) 8 GPa. The *b* and *c*-axis of the original orthorhombic crystal is marked. Note the large contraction in the *b*-direction in (c). Helium freezes at 11.8 GPa at room temperature and is a gas until then.

ambient conditions crystallizes in these structures⁹, (ii) the observed splitting of the high frequency Raman peak (see Figure 5) representing the symmetric stretch is in accord with the splitting of this mode in both the low-clino and ortho-enstatite¹¹, (iii) finally, the quenchability shows that the pyroxene chain is established in the blue-phase: A change from orthorhombic (Pbmm) CuGeO_3 to pyroxene-type structure must involve a 180° flipping of alternate GeO_4 tetrahedra, to convert the 'einer' chain to 'zweier' type chain and this transition involves breaking of bonds and flipping of the CuO_6 octahedra as well. Once this happens under high pressure, the reverse transition would be kinetically impeded. The observed quenchability of the blue phase and its reversion, only when heated to 600°C is consistent with the above expectation.

Our optical observations in polarized light indicate that the single crystal nature is preserved in the blue phase. Hence, it should be possible to carry out single crystal X-ray diffraction on quenched blue phase and the structural prediction verified. In this phase, the spin-Peierls transition should be absent because of the change in the Cu^{2+} array due to conversion to the 'zweier' type chain.

Once the essential ingredient for the pyroxene structure is established, namely the 'zweier' chain, the behavior of MgSiO_3 under pressure could be used as a guide. It has been shown that both clino-enstatite (MgSiO_3)¹² and ferrosilite (FeSiO_3)¹³ transform under high pressure to a high pressure $C2/c$ phase near 7 GPa and 1.75 GPa, respectively. This phase is denser by about 3% compared to the original phase¹³. The transitions in both

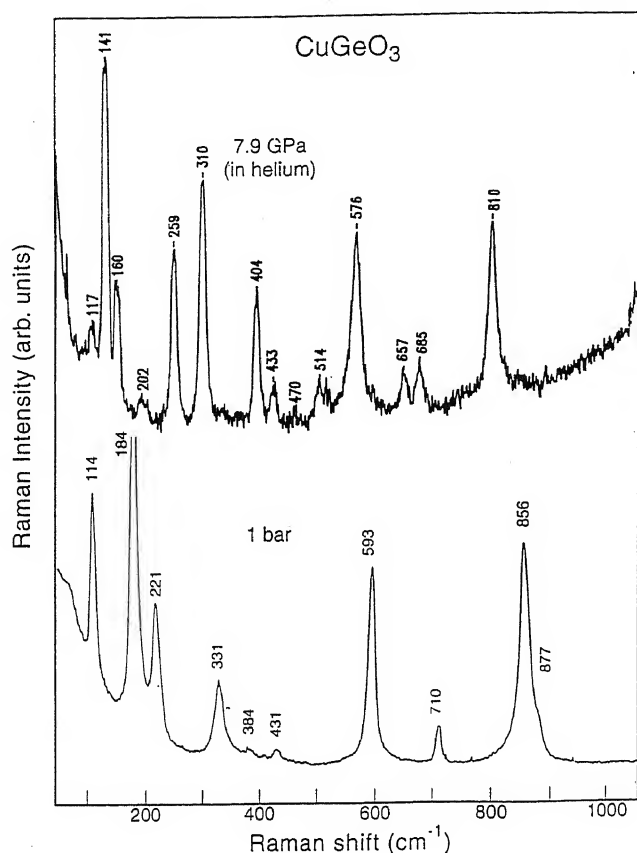


Figure 4. Raman spectrum of single crystal sample of orthorhombic CuGeO_3 at ambient pressure (bottom) and 7.9 GPa, after the phase transition to Phase II. Pressure medium was helium gas. The spectrum is identical to that obtained with methanol/ethanol mixture, as medium.

MgSiO_3 and FeSiO_3 are reversible. The low to high-pressure clino-enstatite transition involves a change from two symmetrically-distinct tetrahedral chains rotated in opposite directions to one symmetrically-distinct tetrahedral chain. We suggest that a similar phase transition occurs in the green phase of CuGeO_3 . Its reversibility and the Raman spectral feature support this interpretation.

The pale green phase-phase II transition occurs only when the pressure medium is *strictly hydrostatic* and not otherwise, and is a very unusual behaviour. For this phase transition, intercalation by the pressure medium (alcohol) was suggested³. However, the occurrence of the very same transition with helium gas also, throws some doubt on the intercalation idea. The presence of a single strong Raman peak in the ν_s (Ge-O-Ge) and ν_s (Ge-O⁻) mode region (see Figure 1) is a strong indication that the 'einer' chain feature is preserved in this phase.

All the evidence we have accumulated so far point to the fact that this Phase I to II transition is abrupt and cannot involve any bond breaking. A sliding or partial rotation of the chain must occur in the structure, to cause the crystal to contract abruptly in the b -axis direction. If the crystal is constrained by a solid pressure medium, this contraction will be impeded and the transition suppressed. When this happens, the competing phase transition to the pyroxene structure takes over, and the 'einer' chain turns into pyroxene-type 'zweier' chain, to minimize the free energy of the compressed system, triggering the blue and then the green phase sequence. Therefore, the freedom for the b -axis to contract or expand is a stringent requirement for I to II phase transition to occur. This would explain the extreme sensitivity of the transition to the hydrostaticity of the medium. These aspects suggest that phase II might be a strongly-coupled ferroelastic system. Usually a ferroelastic transition is coupled to a ferroelectric transition also. One of the sure indications for the latter would be the presence of ferroelectric domains. However, we do not observe any domains in the high pressure phase II when examined in crossed polarization under the microscope. But we believe that the observed jumping of the crystal inside the diamond cell at the transition when helium is used as a pressure medium is significant, and may be connected with the development of electric charge in the crystal due to the sudden compression in the b -axis direction of the crystal. That a ferroelectric crystal when suddenly compressed can get charged is well known. Therefore, it may be that this high pressure phase II of CuGeO_3 is ferroelectric-ferroelastic. Such a coupled situation is known, as is exemplified in the β' -phase of $\text{Tb}_2(\text{MoO}_4)_3$ (ref. 14).

The high pressure behaviour of CuGeO_3 has many interesting facets. The elucidation of the structural features of the high pressure phases by single crystal X-ray diffractions is an inviting challenge to the crystallographer. Once the symmetry and space groups are determined, the interpretation of the vibrational spectra could lead to further insights into the high pressure behaviour. The anomalous b -axis compression carries the key to understand pressure-induced instabilities, just as it is significantly connected to the spin-Peierls instability at low temperatures. The large b -axis contraction at the I to II transition and the extreme sensitivity of the latter to the nature of applied stress suggests a strongly-coupled ferroelastic phase for phase II, with the possibility of ferroelectric behaviour in addition. Application of pressure is a route to prepare a new polymorph of CuGeO_3 , namely, the blue phase at ambient pressure. The properties of this would be of interest. The colours of the phases are rooted in the electronic structure of Cu^{2+} , a $3d^9$ Jahn-Teller ion. A deeper understanding of the absorption features could connect, Jahn-Teller distortion, structure and absorption. Finally, the possibility of

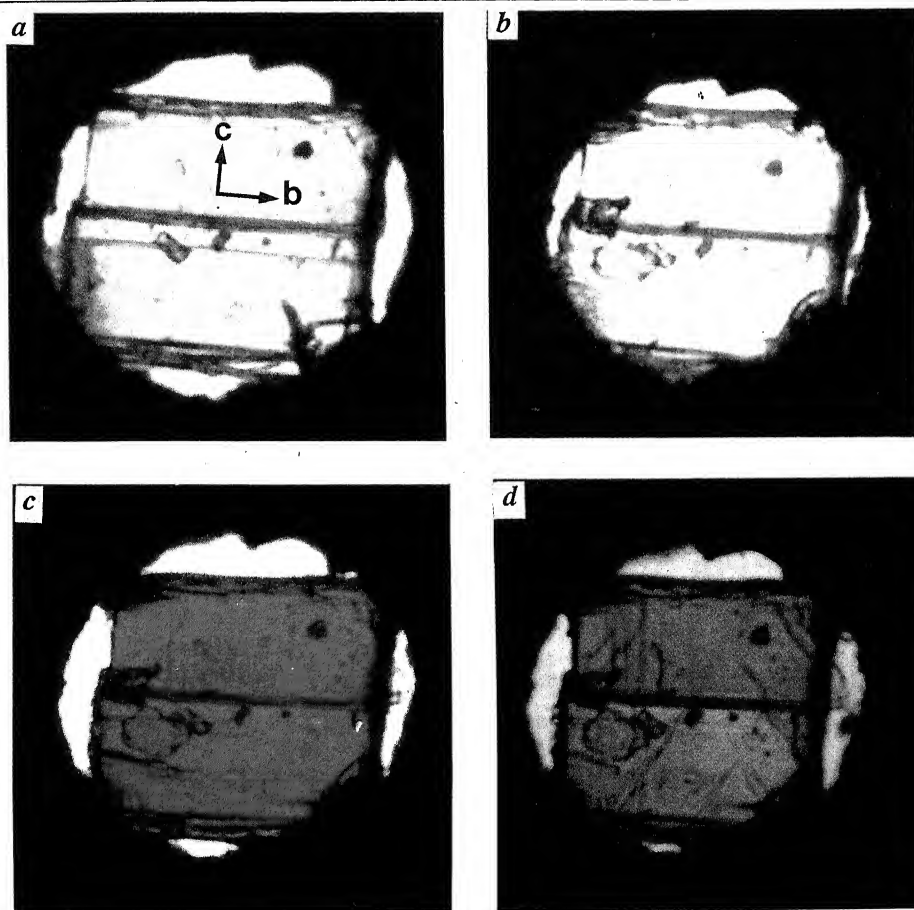


Figure 5. Colour photomicrographs of single crystal CuGeO_3 at four different pressures. Pressure medium argon (a) 1 GPa (b) 5 GPa (c) 4 GPa and (d) 11 GPa. (b) and (c) taken on the pressure release cycle. The b and c-axis of the original orthorhombic Pbnm phase marked.

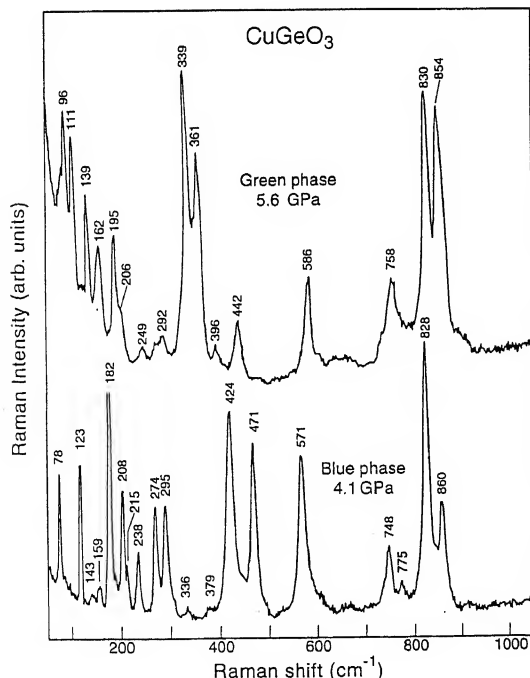


Figure 6. The Raman spectrum of CuGeO_3 single crystal sample in the blue-phase at 4.1 GPa, green-phase at 5.6 GPa, obtained on the pressure release cycle with 514.5 nm excitation. Note the splitting of the high frequency peaks (symmetric stretch).

phase transitions to garnet, ilmenite and perovskite structure exists, in analogy with the behaviour of other germanates and silicates.

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Synthesis and anticancer activity of new derivatives of podophyllotoxin

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A series of analogues of etoposide (VP-16,1), the C-4 alkylamino-substituted 4'-dimethyl-epipodophyllotoxins (4a-g), have been synthesized and studied for their activity to inhibit L1210 and KB cells *in vitro*. Compounds 4a, 4b, 4c and 4f are as potent or more potent than VP-16 in their inhibition of both L1210 and KB cells.

THE clinical efficacy and intriguing mechanism of podophyllotoxin-derived glucoside, etoposide (VP-16, 1), has greatly stimulated interest in the synthesis of new active analogues of podophyllotoxin¹⁻⁶. The approach to modify 1 based on replacement of the glucose moiety with an amino sugar has led to some highly active analogues³, suggesting that β -anomeric configuration was indispensable for the antitumour activity and that the amino substituent of the sugar moiety was important for increasing the activity. Changes in the 4 β -glycosyl group are also of interest for simplified structure which might retain the activity of 1 and its amino glucoside analogues, and be accessible to practical industrialization. In the previous papers^{4,7,8}, we reported the synthesis of an amino nitroxyl spin-labeled analogue of 1, GP-7(2), which exhibited superior pharmacological properties to 1. A series of 4 β -alkylamino and arylamino

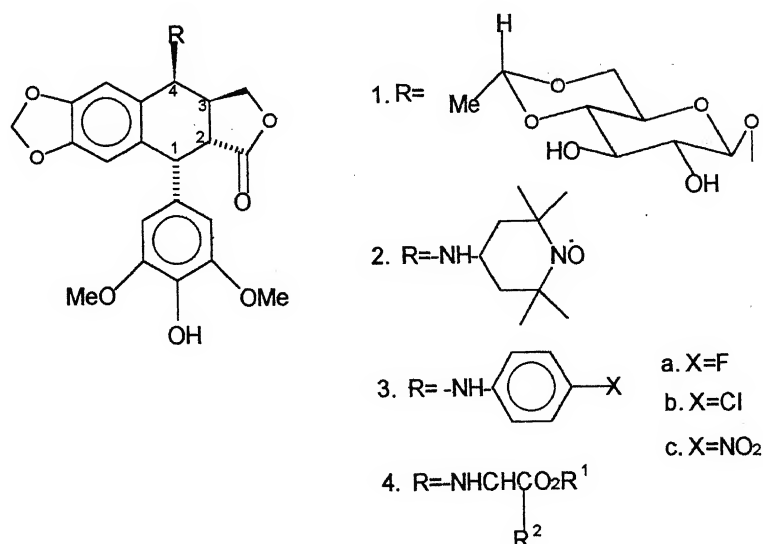
derivatives of 4'-demethylepipodophyllotoxin, such as 3a-c, have also demonstrated by the strong antitumour activity that considerable simplification in the sugar structure might be permitted so long as the amino group was retained^{5,6,9}. These findings prompted us to change the C-4 glucose moiety in VP-16 to a configurationally similar amino-acid ester, and to synthesize seven new derivatives of podophyllotoxin (4a-g).

4 β alkylamino derivatives of 4'-demethylepipodophyllotoxin (4a-g) were synthesized by direct nucleophilic substitution (SN1) of appropriate L-amino-acid ester (7a-g) with 4 β -bromo-4'-demethyl-4-deoxypodophyllotoxin (6) resulting from podophyllotoxin (5). The bulky C-1 α pentant aromatic ring dictates the substitution to be stereoselective in yielding the C-4 β alkylamino isomers as the major products. In some cases, the C-4 α isomers were also observed. The assignment of the configuration at C-4 for compounds 4a-g was based on the difference of $J_{3,4}$ coupling constants. The C-4 β -substituted compounds 4a-g have a $J_{3,4} \approx 4.0$ Hz as seen in 1 and 3 (refs. 5, 6), due to a *cis* relationship between H-3 and H-4. The C-4 α substituted derivatives, however, have a $J_{3,4} \geq 10.0$ Hz as H-3 is *trans* to H-4 (ref. 6).

We have tested the inhibitory activities of compounds 4a-g against leukaemia L1210 and KB cells *in vitro*. ID₅₀ values of compounds 1 and 4a-g are 0.40, 0.28, 0.42, 0.38, 0.70, 1.60, 0.42 and 1.25 μ M for L1210 cells, and 0.22, 0.20, 0.10, 0.18, 0.56, 0.84, 0.28 and 1.00 μ M for KB cells, respectively. Therefore, compounds 4a, 4b, 4c and 4f are as potent or more potent than VP-16 in their inhibition of both L1210 and KB cells. These results demonstrate the possibility of considerable simplification in the sugar structure of 1 and suggest further elaboration of the 4 β -amino substituent to optimize the structure of this class of anticancer compounds. Further study for anticancer activity of synthesized compounds is in progress.

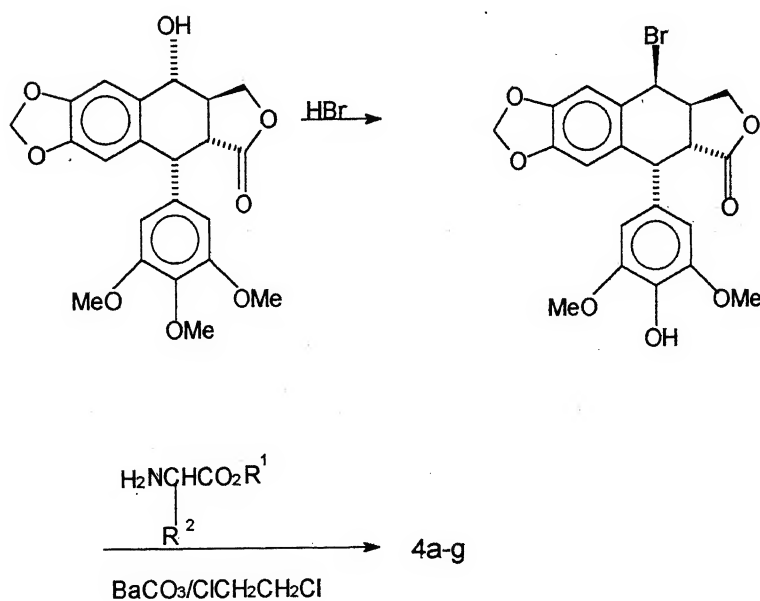
All melting points were taken on Yanaco melting point apparatus and uncorrected. IR spectra were obtained on a Nicolet-5DX spectrophotometer, and ¹HNMR spectra were obtained by using either a Bruker AM-400 or JMS-FX-90Q NMR spectrometer. All chemical shifts are reported in ppm from TMS. Elemental analysis were taken on a YANACO-CHN-CODER MT-3 instrument. MS analysis were determined on a VG-7070E-HF instrument at 70 eV.

A solution containing 4 β -bromo-4'-demethyl-4-deoxypodophyllotoxin (6) (ref. 1) (1.5 mmol), anhydrous barium carbonate (2.0 mmol), and the appropriate L-amino-acid ester (7a-g) (2.0 mmol) in 20 ml of dry 1,2-dichloroethane under nitrogen was stirred overnight at room temperature. The reaction mixture was filtered, diluted with ethyl acetate, washed with water, dried, and purified via column chromatography (50 g of silica gel with dichloromethane-acetone 8:1 as eluant). Yields ranged from 18 to 47%.



a. $R^1 = \text{Et}, R^2 = \text{H}$; b. $R^1 = \text{Me}, R^2 = \text{H}$; c. $R^1 = R^2 = \text{Me}$; d. $R^1 = \text{Me}, R^2 = -\text{CHMe}_2$
 e. $R^1 = \text{Me}, R^2 = -\text{CH}_2\text{Ph}$; f. $R^1 = \text{Me}, R^2 = -\text{CH}_2\text{Ph-OH(p)}$; g. $R^1 = \text{Et}, R^2 = -\text{CH}_2\text{CHMe}_2$

Scheme 1.



Scheme 2.

Compound 4a: m.p. 230–231°C; MS m/z $[M]^+$ 485; IR (KBr) 3500 (OH), 3427 (NH), 1762 (lactone), 1734 (ester), 1614, 1515 and 1483 (aromatic C=C) cm^{-1} ; ^1H NMR (CDCl_3), δ 7.04 (s, 1H, H-5) 6.34 (s, 1H, H-8), 6.15 (s, 2H, H-2', 6'), 5.87 (s, 2H, OCH_2O), 4.70 (s, 1H, OH), 4.40 (d, $J = 5.0$ Hz, 1H, H-1), 4.22–3.96 (m, 4H, H-11 and CO_2CH_2), 3.82 (d, $J = 4.0$ Hz, 1H, H-4), 3.64

(s, 6H, 3', 5'- OCH_3), 3.30 (s, 2H, NHCH_2), 3.24 (dd, $J = 5.0, 14.0$ Hz, 1H, H-2), 2.75 (m, 1H, H-3), 1.92 (s, 1H, NH), 1.24 (t, $J = 7.2$ Hz, 3H, CH_2CH_3). Anal. calculated for $\text{C}_{25}\text{H}_{27}\text{NO}_9$, C 61.85, H 5.61, N 2.89; found C 61.83, H 5.65, N 2.88.

Compound 4b: m.p. 227–228°C; MS m/z $[M]^+$ 471; IR (KBr) 3500 (OH), 3420 (NH), 1760 (lactone), 1734

(ester), 1610, 1511 and 1485 (aromatic C=C) cm^{-1} ; ^1H NMR (CDCl_3), δ 7.00 (s, 1H, H-5) 6.39 (s, 1H, H-8), 6.21 (s, 2H, H-2', 6'), 5.91 (m, 2H, OCH_2O), 5.10 (s, 1H, 4'-OH), 4.49 (d, $J = 5.0$ Hz, 1H, H-1), 4.28 (m, 2H, H-11), 3.89 (d, $J = 4.0$ Hz, 1H, H-4), 3.72 (s, 3H, CO_2CH_3), 3.65 (s, 6H, 3', 5'- OCH_3), 3.37 (s, 2H, NHCH_2), 3.29 (dd, $J = 5.0$ Hz, 14.0 Hz, 1H, H-2), 2.99 (m, 1H, H-3), 2.20 (s, 1H, NH). Anal. calculated for $\text{C}_{24}\text{H}_{25}\text{NO}_9$, C 61.14, H 5.34, N 2.97; found C 61.17, H 5.31, N 3.01.

Compound 4c: m.p. 214–215°C; MS m/z $[\text{M}]^+$ 485; IR (KBr) 3435 (OH, NH), 1772 (lactone), 1742 (ester), 1612, 1515 and 1483 (aromatic C=C) cm^{-1} ; ^1H NMR (CDCl_3), δ 7.02 (s, 1H, H-5) 6.38 (s, 1H, H-8), 6.22 (s, 2H, H-2', 6'), 5.85 (s, 2H, OCH_2O), 5.12 (s, 1H, 4'-OH), 4.42 (d, $J = 4.8$ Hz, 1H, H-1), 4.20–4.00 (m, 2H, H-11), 3.92 (d, $J = 4.0$ Hz, 1H, H-4), 3.78 (s, 3H, CO_2CH_3), 3.70 (s, 6H, 3', 5'- OCH_3), 3.65 (q, $J = 6.8$ Hz, 1H, NHCH), 3.13 (m, 1H, H-2), 2.80 (m, 1H, H-3), 2.06 (s, 1H, NH), 1.15 (d, $J = 6.8$ Hz, 3H, CHCH_3). Anal. calculated for $\text{C}_{25}\text{H}_{27}\text{NO}_9$, C 61.85, H 5.61, N 2.89; found C 61.90, H 5.62, N 2.84.

Compound 4d: m.p. 178–179°C; MS m/z $[\text{M}]^+$ 513; IR (KBr) 3470 (OH, NH), 1770 (lactone), 1736 (ester), 1610, 1508 and 1482 (aromatic C=C) cm^{-1} ; ^1H NMR (CDCl_3), δ 7.00 (s, 1H, H-5) 6.39 (s, 1H, H-8), 6.23 (s, 2H, H-2', 6'), 5.83 (ABq, $J = 1.0, 4.5$ Hz, 2H, OCH_2O), 5.00 (s, 1H, OH), 4.38–4.22 (m, 3H, H-1 and H-11), 3.90 (d, $J = 4.0$ Hz, 1H, H-4), 3.82 (s, 3H, CO_2CH_3), 3.77 (s, 6H, 3', 5'- OCH_3), 3.69 (d, $J = 7.4$ Hz, 1H, NHCH), 3.16 (dd, $J = 5.0, 12.0$ Hz, 1H, H-2), 2.80 (m, 1H, H-3), 2.30 (m, 1H, CHMe_2), 2.21 (s, 1H, NH), 1.10 (d, $J = 7.4$ Hz, 6H, $\text{CH}(\text{CH}_3)_2$). Anal. calculated for $\text{C}_{27}\text{H}_{31}\text{NO}_9$, C 63.15, H 6.08, N 2.73; found C 63.10, H 6.05, N 2.72.

Compound 4e: m.p. 170–172°C; MS m/z $[\text{M}]^+$ 561; IR (KBr) 3500 (OH), 3380 (NH), 1776 (lactone), 1726 (ester), 1612, 1516 and 1485 (aromatic C=C) cm^{-1} ; ^1H NMR ($\text{DMSO}-d_6$), δ 7.35 (m, 5H, C_6H_5), 6.89 (s, 1H, H-5), 6.55 (s, 1H, H-8), 6.34 (s, 2H, H-2', 6'), 5.90 (s, 2H, OCH_2O), 5.41 (s, 1H, OH), 4.44 (d, $J = 8$ Hz, 1H, H-1), 4.38–4.09 (m, 3H, H-11, NH), 3.90 (d, $J = 4.2$ Hz, 1H, H-4), 3.70 (s, 3H, CO_2CH_3), 3.68 (s, 6H, 3', 5'- OCH_3), 3.64 (t, $J = 7.0$ Hz, 1H, NHCH), 3.32 (d, $J = 7.0$ Hz, 2H, CH_2Ph), 3.10 (m, 2H, H-2, 3). Anal. calculated for $\text{C}_{31}\text{H}_{31}\text{NO}_9$, C 66.30, H 5.56, N 2.49; found C 66.38, H 5.58, N 2.47.

Compound 4f: m.p. 197–198°C; MS m/z $[\text{M}]^+$ 577; IR (KBr) 3450 (OH, NH), 1775 (lactone), 1734 (ester), 1610, 1505 and 1480 (aromatic C=C) cm^{-1} ; ^1H NMR ($\text{DMSO}-d_6$, D_2O exchange), δ 6.90 and 6.70 (d, $J = 8.3$ Hz, 4H, C_6H_4), 6.82 (s, 1H, H-5), 6.51 (s, 1H, H-8),

6.26 (s, 2H, H-2', 6'), 5.95 (s, 2H, OCH_2O), 4.48 (d, $J = 5.0$ Hz, 1H, H-1), 4.37 (m, 2H, H-11), 3.95 (s, 3H, CO_2CH_3), 3.83 (d, $J = 4.1$ Hz, 1H, H-4), 3.75 (s, 6H, 3', 5'- OCH_3), 3.67 (t, $J = 7.0$ Hz, 1H, NHCH), 3.30 (d, $J = 7.0$ Hz, 2H, CH_2Ph), 3.18–3.11 (m, 2H, H-2, 3). Anal. calculated for $\text{C}_{31}\text{H}_{31}\text{NO}_{10}$, C 64.46, H 5.41, N 2.43; found C 64.31, H 5.39, N 2.44.

Compound 4g: m.p. 130–131°C; MS m/z $[\text{M}]^+$ 541; IR (KBr) 3443 (OH, NH), 1772 (lactone), 1733 (ester), 1605, 1503 and 1485 (aromatic C=C) cm^{-1} ; ^1H NMR (CDCl_3), δ 6.94 (s, 1H, H-5), 6.46 (s, 1H, H-8), 6.19 (s, 2H, H-2', 6'), 5.91 (m, 2H, OCH_2O), 5.32 (s, 1H, OH), 4.47 (d, $J = 5.0$ Hz, 1H, H-1), 4.28–4.00 (m, 4H, H-11, CO_2CH_2), 3.80 (d, $J = 4.2$ Hz, 1H, H-4), 3.74 (s, 6H, 3', 5'- OCH_3), 3.66 (t, $J = 7.0$ Hz, 1H, NHCH), 3.30 (m, 1H, H-2), 2.94 (m, 1H, H-3), 2.18 (s, 1H, NH), 1.85 (m, 3H, CH_2CHMe_2), 1.28 (t, $J = 7.2$ Hz, 3H, CH_2CH_3), 0.97 (d, $J = 6.6$ Hz, 6H, $\text{CH}(\text{CH}_3)_2$). Anal. calculated for $\text{C}_{29}\text{H}_{35}\text{NO}_9$, C 64.31, H 6.51, N 2.59; found C 64.21, H 6.50, N 2.62.

Drugs were dissolved in Me_2SO at a concentration of 20 mM as the stock solution and diluted before use with H_2O to the desired concentration of each drug. The cytotoxicity was determined *in vitro* using L1210 and KB cells grown in RPMI No. 1640 medium supplemented with fetal calf serum according to the published procedure^{9,10}.

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Contributions of oxide film and bacterial metabolism to the ennoblement process: Evidence for a novel mechanism

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Biofilms were grown in laboratory conditions by exposing a range of alloys such as stainless alloys (grade-2 titanium, 6XN, 316L, 904L, Seacure, C276, platinum), aluminium 2S, aluminium 6061, chromium, nickel, molybdenum, copper and cupro-nickel (90:10) to natural pond water. On the basis of photo-electrochemical studies, the oxide films on the above materials were classified as n or p type semiconductors. Alloys having an overlayer of n-type semiconducting oxide film only exhibited a substantial positive shift of corrosion potential. Capacitance measurements were employed to analyse the changes in donor concentration within the oxide film during the ennoblement process. Bacterial and chemical constituents of the biofilm were also analysed. Results of the study strongly suggest that the mechanism of ennoblement is linked to the bacterial removal of excess 'anions' and 'cations' from the oxide film.

BIOFILMS from fresh as well as sea waters usually shift the open circuit potential of stainless steels by several hundred millivolts¹⁻⁵. Johnsen and Bardal⁶ suggested that this process of ennoblement is linked to organo-metallic catalysis of the oxygen reduction reaction. Alternatively, the groups of Dexter hypothesized that the ennoblement is caused by a decrease in pH^{2,7,8} at the metal surface by respiring organisms and by the production of peroxide³. Maruthamuthu *et al.*⁴ proposed that the ennoblement is not an effect of low pH but rather linked to neutral pH conditions. Eashwar *et al.*⁵ explained that the ennoblement was caused by anodic passivation following neutral pH. Eashwar and Maruthamuthu⁹ have also pointed out that siderophores produced by bacteria could be important to ennoblement.

Most recently, Maruthamuthu *et al.*¹⁰ proposed an adsorbed inhibitor theory which emphasized an importance for oxide film in the process of ennoblement. In the

present investigation, the contributions of oxide film and bacterial metabolism are examined.

The tests were conducted on grade-2 titanium, stainless steels (316L, 904L, Seacure), super alloys (C-276, 6XN), platinum, aluminium 2S, aluminium 6061, chromium, nickel, molybdenum, copper and cupro-nickel (90:10). The samples were pickled in appropriate acids¹¹, polished with 400 grade emery, degreased in acetone and rinsed in distilled water prior to all tests. The compositions of the different alloys used are given in Table 1.

For potential measurements, the various alloys were exposed to freshly-sampled pond water in laboratory conditions. Open circuit potentials (OCP) were measured with respect to a saturated calomel electrode (SCE) using a 4 digit high impedance multivoltmeter (HIL 2605). Coupons of 5 cm × 2 cm size were used for potential measurements.

The impact of dissolved oxygen concentration (DO) on potential of titanium was also examined by lowering the dissolved oxygen concentration by the addition of sodium sulphite to the filter-sterilized (0.2 micron) water.

For photopotential measurements, two identical electrodes of each metal/alloy were polished to a mirror finish, degreased with trichloroethylene and lacquered to obtain a geometrical area of 1 cm². An all-PVC cell (300 ml capacity) consisting of two compartments, separated by a thin perforated sheet, was so designed that one electrode could always be kept in the dark while the other could be irradiated through a quartz window as and when required. In all the above systems, biofilms were grown on the coupons by renewing the natural pond water every day. Depending upon the direction of the photopotential, the overlaying oxide film was identified as n- or p-type semiconductor.

Capacitance measurements were carried out using a conventional three-electrode electrochemical system with platinum foil as the auxiliary electrode and saturated calomel as the reference electrode using PAR electrochemical impedance system. Two 904L coupons exposed to natural and filtered pond water for 40 days were used as the working electrodes.

The nutrient content of the biofilm was analysed at various time intervals after immersion. The biofilm was scrapped using a uniform edged sterilized knife and collected in 100 ml triple distilled water. This sample was used for estimating dissolved nitrite, nitrate, total phosphorus and inorganic phosphates according to Grasshoff *et al.*¹².

Figure 1 shows the open circuit potential (OCP) for 316L stainless steel, chromium, nickel and molybdenum exposed to pond water. It clearly indicates that the OCP of 316L stainless steel alone increases with time to more positive values.

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Table 1. Composition of various stainless steel alloys and grade 2 titanium

Element	904L	AL6XN	Seacure	C-276	316L	TiGr2
Al	—	—	0.027	—	—	—
C	0.018	0.019	0.025	<0.02	0.053	0.01
Cr	19.80	20.30	27.36	15.79	17.56	—
Cu	1.52	0.270	0.10	—	—	—
Fe	Bal	Bal	Bal	5.239	Bal	0.07
Mn	1.55	0.52	0.33	0.42	1.60	—
Mo	4.28	6.25	3.53	15.58	2.21	—
Ni	24.10	23.93	2.02	58.93	11.29	—
P	0.019	0.026	0.025	0.006	0.021	—
S	—	0.0003	0.002	<0.001	0.030	—
Si	0.420	0.39	0.41	0.04	0.86	—
Ti	—	—	0.44	—	—	—
Co	0.270	—	0.10	0.22	0.25	—
N	0.057	0.24	0.019	—	0.040	0.005
W	—	—	—	3.76	—	—
V	—	—	—	0.01	—	—
Ti	—	—	—	—	—	Bal
O	—	—	—	—	—	0.111
H	—	—	—	—	—	0.002

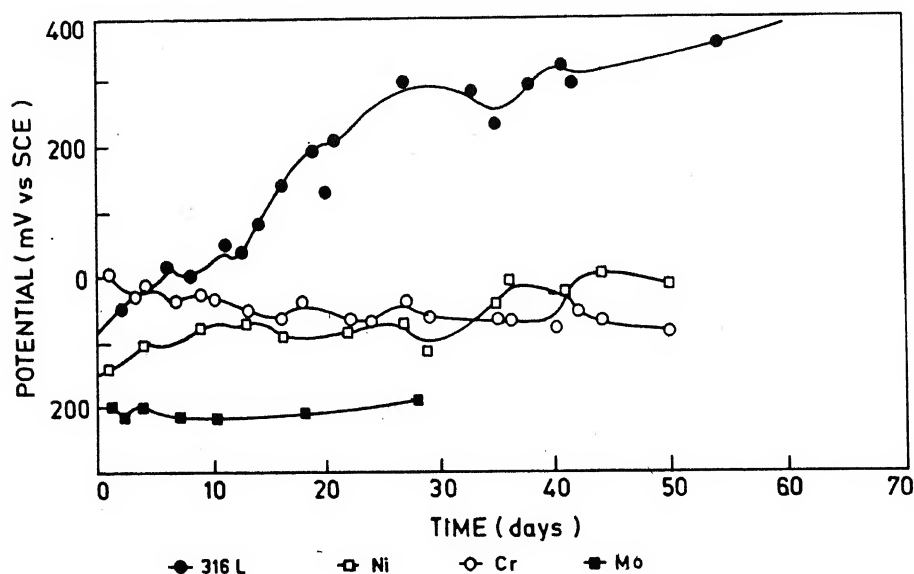


Figure 1. Potentials for 316L SS, nickel, chromium and monel in biotic system.

The OCPs of 6XN, 904L, Seacure and C-276 with time are shown in Figure 2. A large ennoblement is observed for 6XN and the least for C-276.

Table 2 presents the data on the ennoblement range, magnitude of photopotential and semiconducting type of oxide film for various metals/alloys after 32 days of immersion in natural pond water.

The Mott-Schottky ($1/C^2$ vs E) plots for 904L biofilmed electrode and unbiofilmed electrode in pond water are shown in Figure 3. An increase in $1/C^2$ values (i.e., decrease of capacitance) (see ref. 15) is observed on biofilmed specimen as compared to the control specimen. From the capacitance measurement, donor

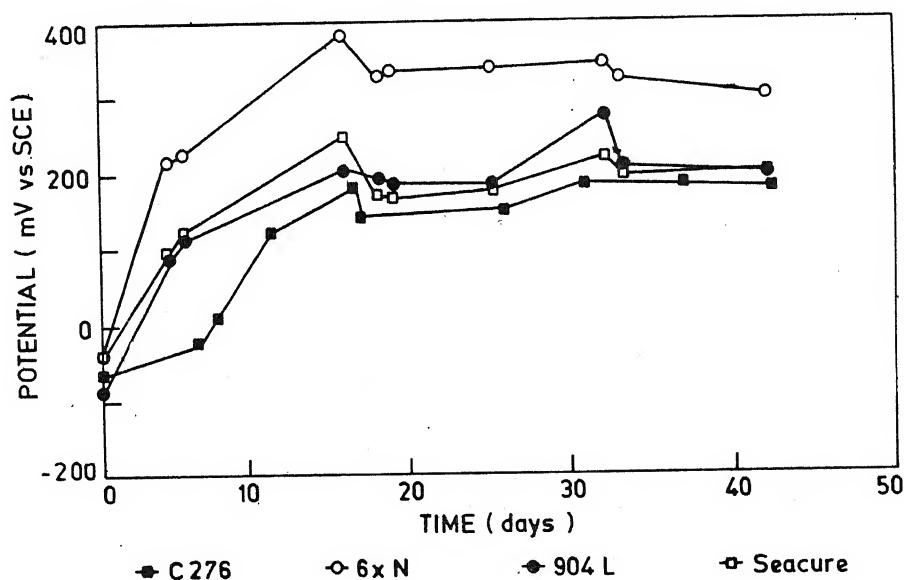
concentrations (at 1000 Hz) as calculated from the slope of the linear region of the curves, are 5.3371×10^{17} for the biofilmed specimen and 1.5929×10^{18} for the bare specimen.

Figure 4 shows the OCP of titanium in filter sterilized deaerated or aerated pond water (pH 8.6 to 9.2) with time. A decrease in dissolved oxygen shifts the potential of titanium to more negative values whereas in deaerated water there is not much fluctuation in OCP.

Figure 5 shows the adsorbed nutrients value of biofilm on titanium. Total phosphorus and nitrate are high in 10 days old biofilm. Estimated nitrite value is low on all days.

Table 2. Data on ennoblement range, amount of photopotential and nature of oxide film for various alloys

Materials	Ennoblement range (mV)	Photopotential	Nature of oxide film
316L SS	300–400	–40	n-type
Chromium	No ennoblement	+10	p-type
Nickel	No ennoblement	+10 – +15	p-type
Molybdenum	No ennoblement	–	Unstable (bluish black)
904L	275–300	–25	n-type
6XN	330–375	–30	n-type
C-276	200	–10 to 13	n-type
Seacure	250	–20	n-type
Titanium	300	–52	n-type
Platinum	450	–50	n-type
Aluminium 2S	No ennoblement	+2	p-type
Aluminium 6061	No ennoblement	+3	p-type
Copper	No ennoblement	+75	p-type
Cupro-nickel	No ennoblement	+60	p-type

**Figure 2.** Potentials for stainless alloys of C276, 6XN, 904L and seacure in biotic system.

In the present study, the photopotentials have been measured to determine the nature of oxide film on various alloys. The photopotential of a metal oxide can be expressed as

$$V_{ph} = KT/(e \ln (N_A/N_D)), \quad (1)$$

where N_A and N_D are the concentrations of acceptor and donor corresponding to those excessive anions and cations and the stoichiometric composition of oxide film^{13,14}. Alloys can be classified as n-type or p-type semiconductors based on the negative or positive shift in potential due to light.

It is seen from Table 2 that a positive shift (ennoblement) in OCP is observed only for metals/alloys having an n-type semiconducting oxide film. Figure 1

explains that while ss 316 shows an ennoblement, the same is not the case for chromium, nickel and molybdenum. This is because chromium and nickel have p-type semiconducting oxide films and molybdenum has an unstable black oxide film. It has to be explained here that even though chromium is present in stainless steel, FeOOH is in outer layer and Cr_2O_3 is in the inner layer¹⁵. The outer layer therefore acts as an n-type oxide and supports the ennoblement process.

The positive shifts in corrosion potential are large for 6XN, 904L and Seacure but least for C-276 (Figure 2). Results in Table 2 indicate that the semiconducting oxide film on C-276 is an n-type, although the alloy contains high percentage p-type inclusions such as nickel, chromium and molybdenum (Table 1). The high per-

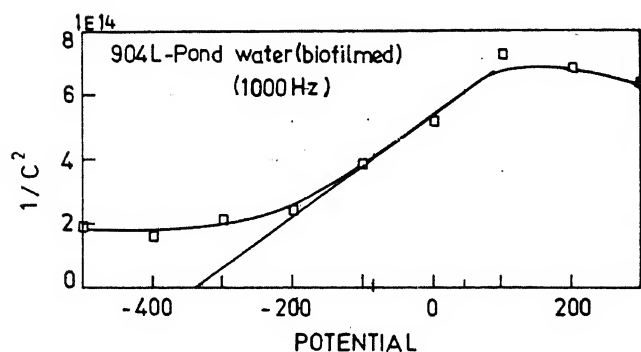
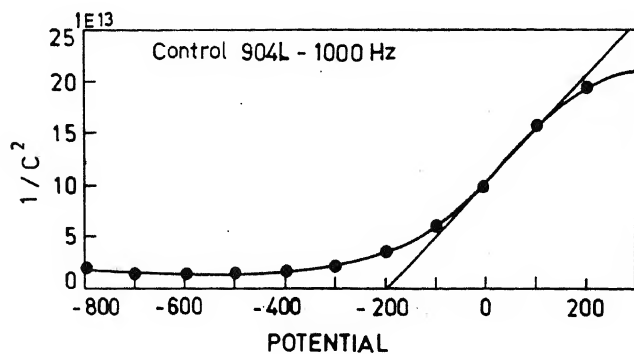


Figure 3. Capacitance vs potential in biotic and abiotic system for 904L at 1000 Hz.

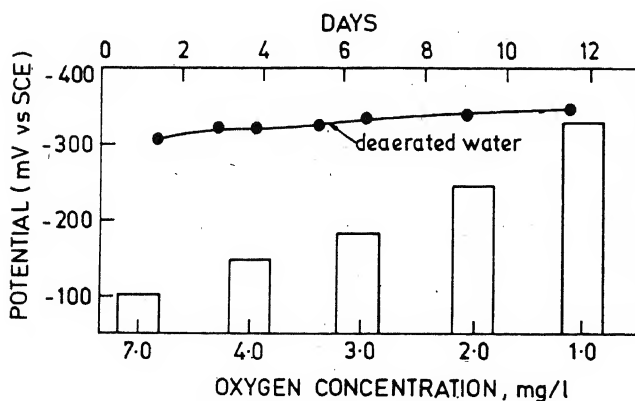


Figure 4. Potential values in various oxygen concentrations.

centage of these inclusions, especially molybdenum¹⁵ might reduce the donor concentration on C-276 alloy and hence be the reason for the least positive shift. But the reason for the high ennoblement range in Delaware waters⁸ and Tuticorin (personal observation) for C-276 needs further exploration. The present observation

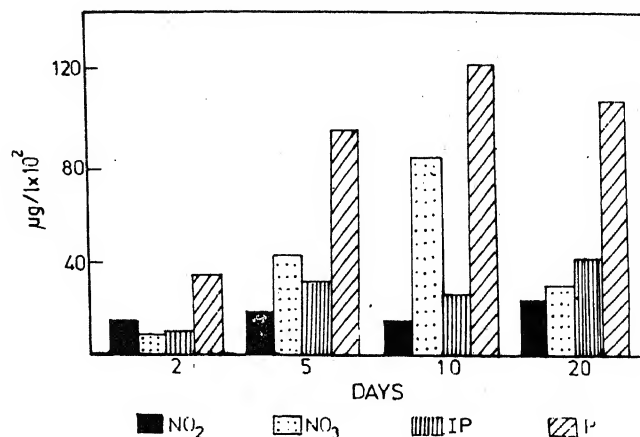


Figure 5. Concentrations of various nutrients in biofilm.

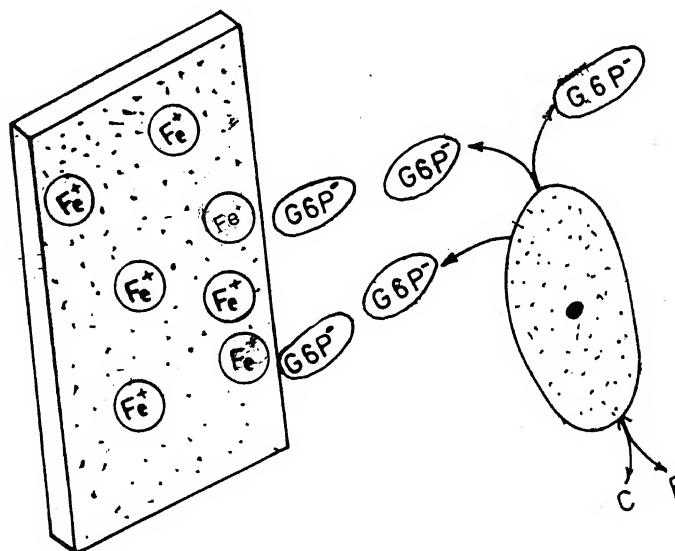


Figure 6. Model for ennoblement mechanism.

suggests that the range of positive shifts for various alloys may depend upon the donor concentration (excess cations) of oxide films. Hence, capacitance measurement has been taken for 904L with and without biofilm.

For a semiconducting/electrolyte interface, the net capacitance C is related to the capacitance of the space charge layer C_{SC} and the capacitance of the Helmholtz layer C_H according to

$$1/C = 1/C_{SC} + 1/C_H \quad (2)$$

Normally, the capacitance associated with the Helmholtz region is very large compared to that of space charge region and therefore the total capacitance C will correspond to C_{SC} . The $1/C^2$ vs potential plots were analysed using the expression for the space charge capacitance of a semiconductor in the depletion region

$$1/C^2 = 2/(\epsilon\epsilon_0 e N_D (V - V_{fb} - KT/q)), \quad (3)$$

where ϵ is the dielectric constant of the film, N_D the donor concentration, V the applied electrode potential, V_{fb} the flat band potential and the remaining symbols have their usual meaning. According to the equation, the donor concentration can be calculated from the slope of the linear region. The calculation of donor concentration requires the knowledge of the dielectric constant of the films.

In the present study, increased oxide film thickness¹⁰ and decreased donor concentration were observed in presence of a mature biofilm. The capacitance values were also less the biofilmed sample as compared to the control. This result may be explained in terms of the production of anions by bacterial metabolism which could reduce the donor concentration of the oxide film by neutralization with excess cations. The results also suggest that the organic nutrients can act as a semiconductor and enhance the thickness of the oxide film.

According to Chao *et al.*¹⁶, thin passive films contain many lattice defects and as shown by Sato *et al.*¹⁷ for passive iron, the density of these defects acting as electron donors decreases with increasing film thickness because the film tends to take a more stable structure. The lower N_D values obtained in neutral solutions confirm that the amorphicity of the passive films decreases with increasing film thickness. Increasing thickness of the oxide film and anion production by biofilm are a continuous and permanent process which can strengthen the oxide film and make it more stable. This mechanism is similar to the observations made by Irhzo¹⁸ on stainless steel in presence of molybdenum anions. The molybdenum anions MoO_4^{2-} neutralize the positive donors of oxide film, decrease the conductivity and possibly repel the chloride adsorption to increase the pitting resistance of stainless steels.

Figure 4 explains the positive shift in OCP at high oxygen levels. Viera *et al.*¹⁹ also observed the shift in the noble direction in the case of stainless steel when ozone was present in a cooling water system. The positive shifts in chloride solution were also observed by some authors^{10,20}. These abiotic positive shifts may be explained as the availability of anions (O^- ; Cl^-) may influence the oxide film which may favour the positive shift.

However, Little *et al.*²⁰ have performed oxygen measurements through microprobe to show that the biofilm substratum interface remained virtually oxygen free. Guezennec²¹ clearly showed that an appreciable ennoblement started after 20 days only, when anaerobic bacteria begin to flourish. Further, the sharp influence in corrosion potential was coincident with an increase in the numbers of *Desulphovibrio* and *Desulphatovaculum* species. The potential after a gradual increase up to 50th day, remained unchanged for a further period of 30

days, which was characterized by the domination of these anaerobic species. Recently Eashwar and Maruthamuthu⁹ concluded that anaerobic bacterial activity is to be expected in all biofilms. In the present study also, the ennobled specimens of 316L in the OCP of around +390 were maintained up to 6 months in the freshwater. Hence, it suggests that in presence of biofilm, the oxygen and chloride are not needed for ennoblement.

Generally, the oxide growth may occur by cationic movement outwards from the metal/oxygen interface or by anionic movement inwards²². Hence, the abiotic and biotic shift may be explained by movement of anions/cations of oxide film.

Figure 5 shows that the biofilm contains high amounts of organic phosphate with nitrates and nitrites. Maloney *et al.*²³ have explained the anion exchange mechanism for both gram-positive and negative cells. The bacteria, for maintenance of a physiological carbon : phosphorus ratio (40:1) during the growth of the cell, brings out the too little carbon and too much phosphorus from the cell in the form of glucose-6-phosphate anion (G6P^-) (Figure 6). Bhosle *et al.*²⁴ have recently identified eight major individual sugars like arabinase, fructose, galactose, glucose, mannose, rhamnose, ribose and xylose in micro-fouling material. The proposed mechanism is that the anion of organic phosphate combines with excess cations of n-type oxide film leading to both strengthening of oxide film and a positive shift in the corrosion potential. The 'mixed, biologically produced, organic complex' may act as 'anion', strengthen the 'oxygen starved'²¹ oxide film for long periods of time. Further study is in progress to present more evidence in support of this novel mechanism.

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Defective neurulation in frog embryos exposed to dilute sea water

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Embryos of *Microhyla ornata* were exposed to dilute sea water from early gastrula stage onwards for a period of 48 h. The process of neurulation was studied in control and treated embryos by using optical and scanning electron microscopy. In the treated embryos, the neural folds formed normally in the initial period of exposure and subsequently approached each other. However, they failed to fuse mid-dorsally in the cephalic region. In the posterior half of the embryos, the neural folds fused to form the neural tube. Irrespective of this, the treated embryos continued differentiation of the brain, as was evident from the development of the eyes. Failure of ectodermal cells to cover the neural cells may be related to the dramatic surface modifications induced due to high concentration of cations like Na⁺.

EFFECTS of saline medium on amphibian embryos have been widely studied for various reasons. For example, Ely¹ has studied effects of dilute sea water on embryos and tadpoles of a toad, *Bufo marinus*, and has described the tolerance levels. To find out if acidity of breeding ponds is a limiting factor determining distribution of amphibians in New Jersey, Gosner and Black² have in-

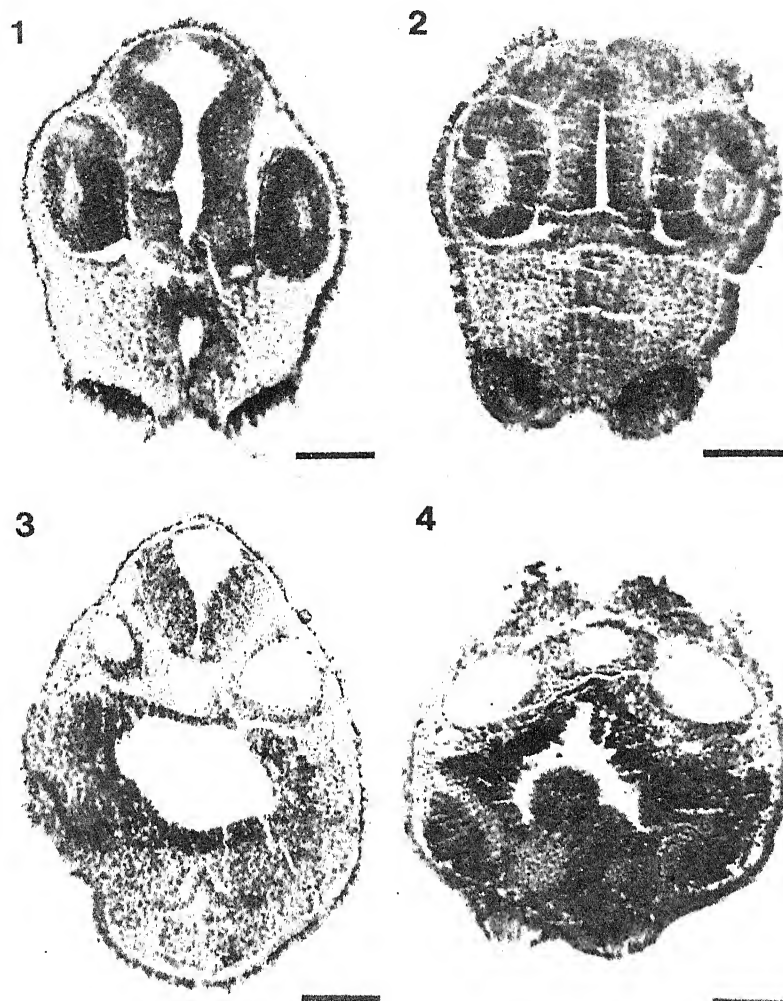
vestigated effects of altered pH and salinity on the embryonic development of several species of frogs. With a view to understanding the ecological relationship of amphibians to brackish water, Ruibal³ has studied effects of salinity on embryos of *Rana pipiens*. Salthe⁴, who was interested in finding out the mechanism of increase in the volume of perivitelline space during early amphibian development, has also reported effects of low pH, various cations and anions on the embryos of *R. pipiens*.

In recent years, Beebee⁵ has studied salt tolerance of the embryos of natterjack toad (*Bufo calamita*) because breeding ponds of these toads are subjected to salt spray and tidal inundation in some coastal areas of Britain. Desiccation of breeding ponds due to irregular rainfall and possibility of tidal inundation of ponds in coastal areas prompted Padhye and Ghate⁶ to investigate salt tolerance of the embryos of *Microhyla ornata*.

While studying the ecology of brackish water population of *R. pipiens* from California, Ruibal³ has made an interesting observation regarding neurulation of the embryos exposed to near-lethal concentration of sea water - it has been mentioned that at salinities above 5‰ the surviving embryos displayed anteriorly open neural groove. Similar effects have latter been photographically documented along with brief histology of defective neural tube⁶.

In this paper we provide additional evidence in the form of histological and scanning electron microscopic (SEM) analyses of defective neurulation in *M. ornata* embryos exposed to dilute sea water. The interesting facts emerging out of this work are: (1) the mechanism of neural tube closure may be different along the anteroposterior axis of the neural tube, (2) exposure to saline medium leads to collapsing of elevating neural folds as well as detachment of nonneural and neural ec-

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Figures 1–4. 1, T. S. control embryo at 24 h (post gastrulation). Note developing eyes and suckers. Neural tube is completely closed and covered over by ectoderm. Scale bar = 100 μ m. 2, T. S. experimental embryo (30% sea water) of the same age as above. Note open neural tube, nonneural ectoderm detaching from the neural cells. Neural folds have, however, approached midline and partially fused (see also Figure 7 for magnified view). Scale bar = 100 μ m. 3, T. S. control embryo (as in Figure 1) through otic capsule region. Again the neural tube is completely closed and has typical morphology. Notochord and otic capsules are well developed. Scale bar = 100 μ m. 4, T. S. experimental embryo of the same age as above. Note highly abnormal neural tube. The ectoderm is widely separated and neural cells are actually falling away. Notochord and otic vesicles are normal. Suckers are also seen on the ventral side. Scale bar = 100 μ m.

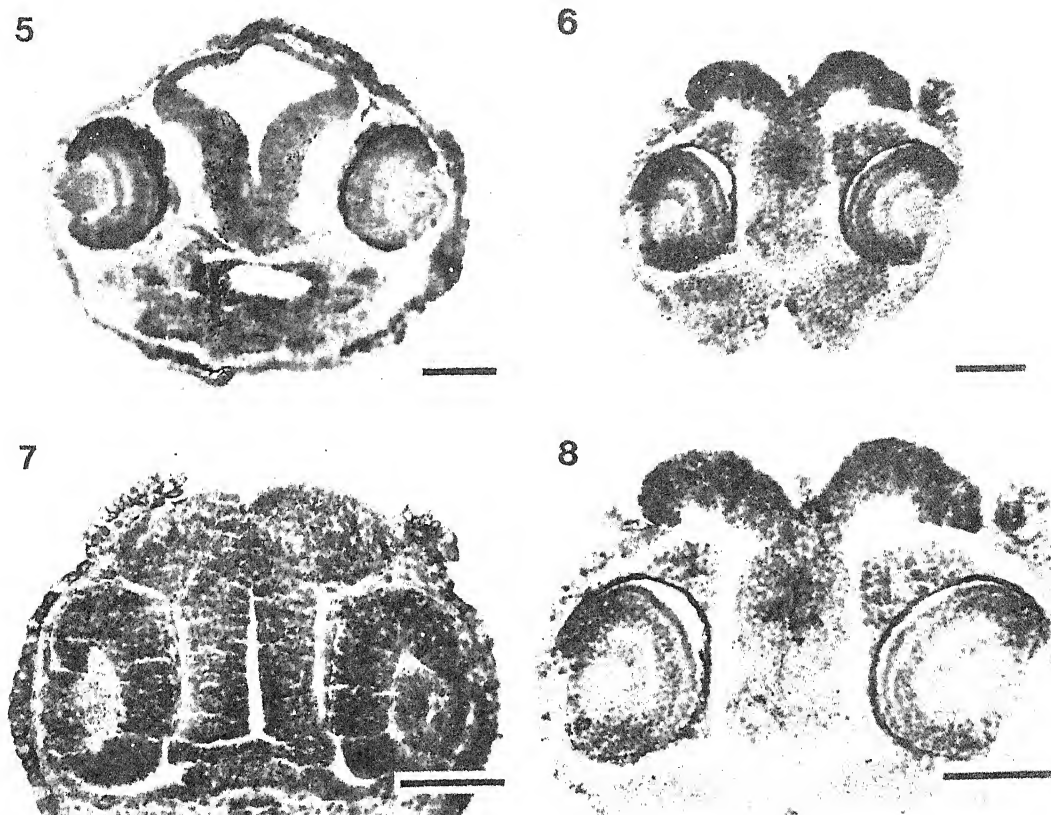
toderm from each other and (3) in spite of defective neurulation, development of eye and otic capsule proceeds normally at least for some time.

Embryos of the frog *M. ornata* were collected from local freshwater ponds, jelly was removed manually using forceps and the embryos in early gastrulation (stage 10 of Gosner⁷) were exposed to different dilutions of sea water according to the methods described earlier⁶. The sea water was collected from an apparently unpolluted site on the west coast and stored in a sealed polypropylene jar at about 10°C until use. It was diluted with distilled water so as to obtain 10%, 20% ... 70% sea water. The sea water used was collected from the west coast during heavy monsoon rains and it had salinity of 24 parts per thousand. The total sodium and potassium con-

centration measured by flame photometry was 7200 mg/l and 250 mg/l, respectively.

Since one of the prominent effects of sea water exposure on embryos was defective neurulation, we carried out histological examination as before⁶. In addition, SEM examination was carried out on whole embryos processed as described by Ghaskadbi⁸.

Sea water at a concentration of 60% and more was found to be rapidly lethal to developing embryos of *M. ornata*. At 60% and 70% concentrations, sea water killed all the exposed embryos within a few hours of exposure. Sea water concentration of 40% and 50% was tolerated by most embryos up to about 48 h. In 30% sea water, the embryos survived up to 72 h while 10% and 20% sea water was tolerated beyond 96 h. The experi-



Figures 5–8. 5, T. S. control embryo at 48 h. Note well-developed typically shaped neural tube, eyes and gut. (To choose the appropriate matching section, we had to include this section which unfortunately has a knife mark.) Scale bar = 100 μ m. 6, T. S. experimental embryo at the same age as above. The neural tissue is abnormally fused in the region between the eyes while it is uncovered by ectoderm dorsally. The ectoderm appears to be completely detached from neural tissue and neural folds are collapsing (see Figure 8, magnified view). Scale bar = 100 μ m. 7 and 8, Slightly magnified views of the sections presented in Figures 2 and 6 respectively. Scale bars = 100 μ m.

ments were terminated at 120 h because the purpose was to see effects on early development.

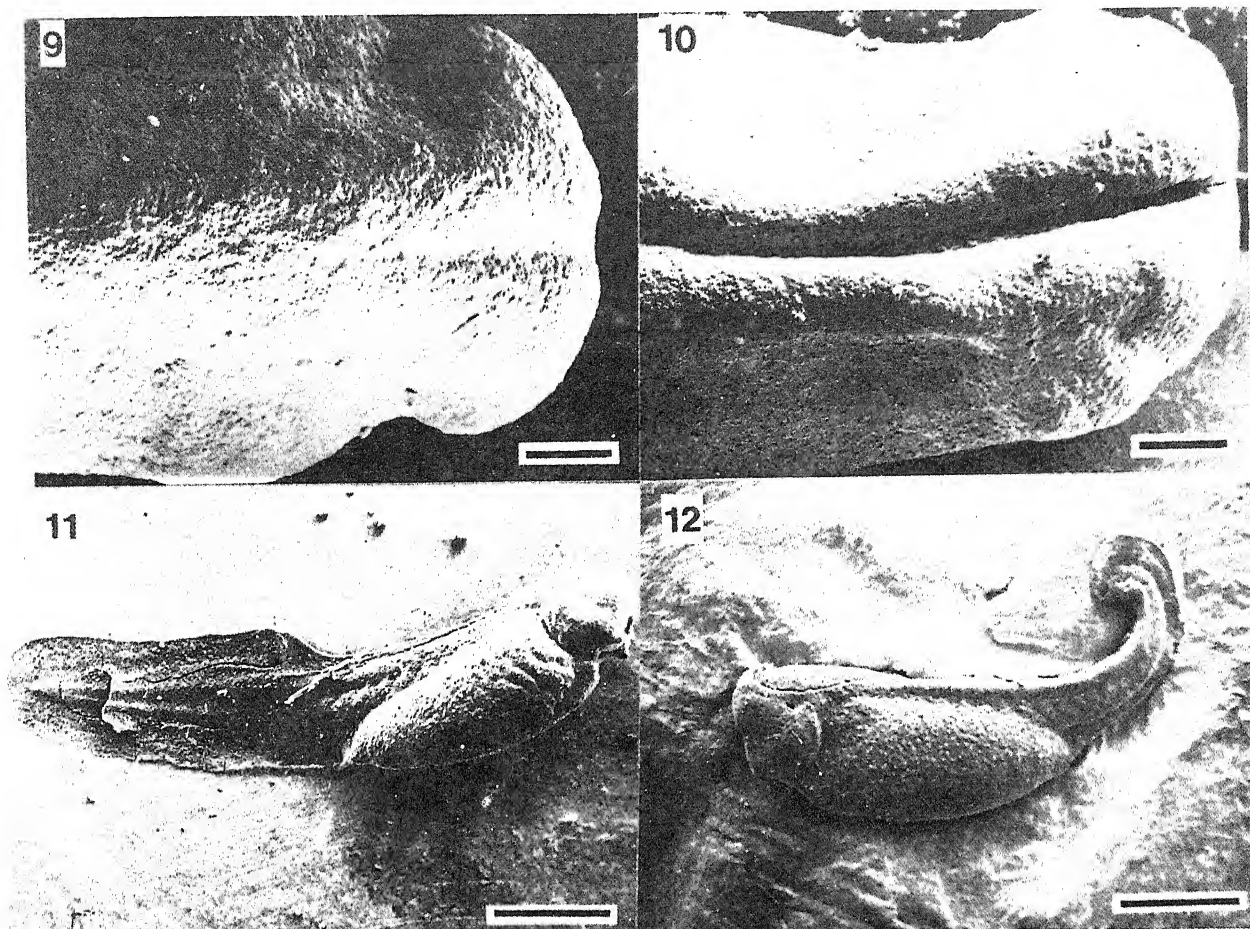
Prominent effects of sea water on embryonic development were observed between 30 and 50% concentration. The effects observed were: failure of the enlargement of perivitelline space, curling of the body axis, reduced percentage of hatching, slight retardation of development and reduced pigmentation. The severity of these effects increased with the increasing percentage of sea water in the medium.

With respect to neural tube development, all embryos exposed to 30–40% sea water showed open neural tube in the head region. In 30% sea water, the embryos were otherwise well-developed and showed normal movement within the vitelline membrane. Most of these abnormal embryos survived for at least 72 h or more. In 40% sea water, however, the embryos were more stunted and, although they also showed open neural tube, most were dead by 48 h. All histological and SEM work was therefore carried out on embryos that survived exposure to 30% sea water.

In 50% sea water, embryonic development was arrested within a few hours of exposure, at about neurula or tail bud stage. Here again the neurulation was not normal. Beyond 50% the embryonic development was arrested as soon as the embryos were exposed, so the event of neurulation could not be observed.

In control embryos, development was quite rapid and normal in all the embryos. There was no mortality or signs of abnormal development in any. By the end of 48 h most control embryos hatched as tadpoles-with-suckers and attached themselves to the wall of the container. In the next 24 hours, eyes and pigmentation developed and the tadpoles were actively swimming. Different regions of the body could be easily observed in these tadpoles (head, trunk, tail).

Comparison of the transverse sections of control and experimental embryos revealed that, in the embryos exposed to 30% sea water, the neural tube was open dorsally in the head region as well as in the anterior part of the trunk region. In the tail region, however, the neural tube had closed. A section passing through the head re-



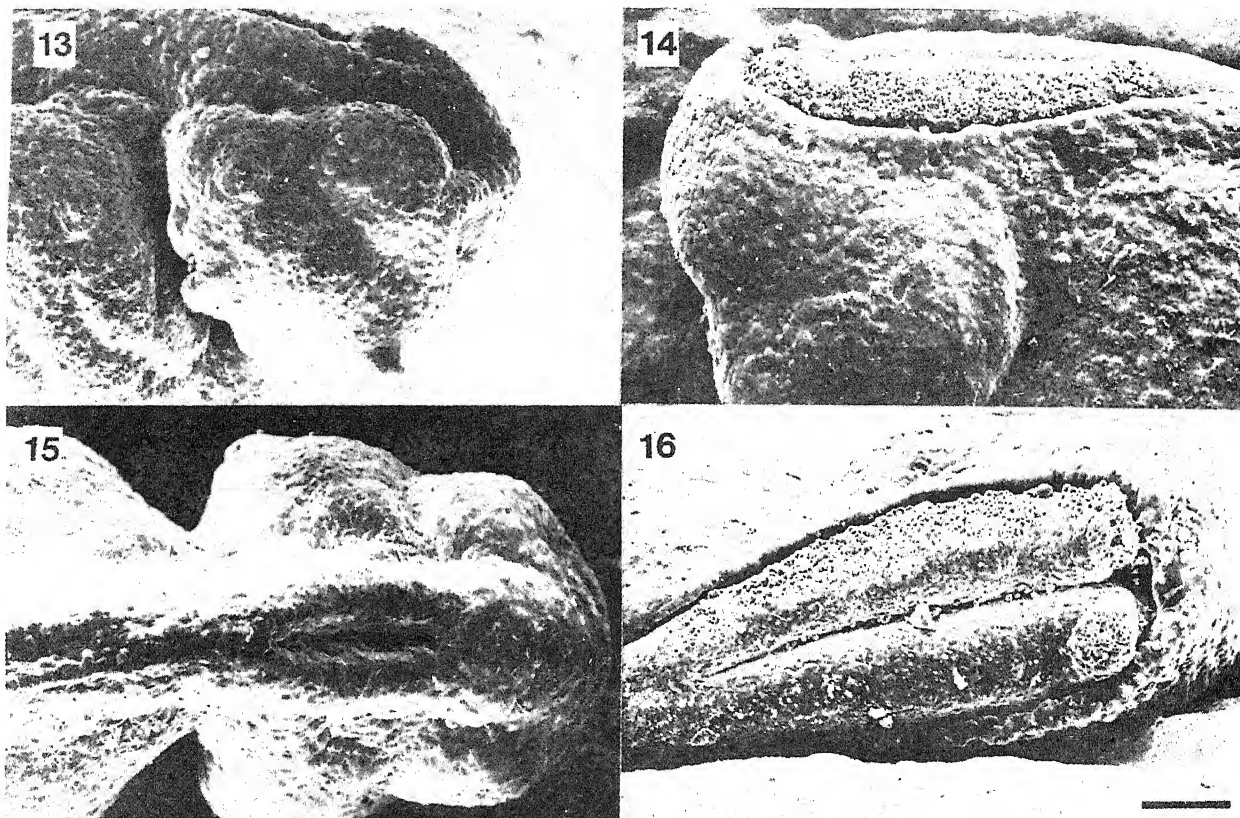
Figures 9-12. 9, Dorsal view control neurula. Note complete closure of the neural folds. Scale bar = 100 μm . 10, Dorsal view of the corresponding experimental embryo. The neural folds have approached towards the midline and there is delay in fusion. At this stage there is no evidence of detachment of nonneural and neural ectoderm. Scale bar = 100 μm . 11, Later view of 24 h (post gastrulation) control embryo. Note head, trunk and tail as distinct regions of the embryo. Suckers and external gills are also visible. Scale bar = 500 μm . 12, Lateral view of the corresponding experimental embryo. The neural tissue in the anterior two third part is not covered by the ectoderm and the tail is curved. Scale bar = 500 μm .

gion of the control embryo showed well-differentiated neural tube; the eyes and suckers were becoming evident at 24 h post gastrulation (Figure 1). A comparable section of the experimental embryo showed dorsally open neural tube with collapsing neural folds. Nonneural ectoderm had not closed over and neural cells were exposed (Figure 2). Sections passing through the otic-capsule region also indicated that neurulation was badly affected in the experimental embryos (Figures 3 and 4). Development of otic (ear) capsules and notochord was apparently unaffected.

At 48 h the control embryo showed well developed neural tube, eyes and gut region (Figure 5). In the case of experimental embryos, however, the neural tube was wide open and highly abnormal. The nonneural ectoderm had failed to close mid dorsally and the neural

tissue formed a fused mass in between the developing eyes (Figure 6). Figures 7 and 8 are magnified views of the embryos shown in the Figures 2 and 6. These two figures (Figures 7 and 8) clearly point out the distorted development of the neural tube. Eye development was found to be nearly normal, however.

Scanning electron microscopy further revealed that neurulation was affected early in the development. At a stage when control embryos had completed neurulation (Figure 9), the experimentals were lagging behind and the neural folds were still approaching the midline (Figure 10). In a few hours it was clear that the neural folds of the experimental embryos were abnormal and did not fuse in the mid dorsal line. After about 24 h from the commencement of the experiment, the control embryos had reached miniature tadpole stage with



Figures 13–16. 13, Magnified view of the head region of control embryo from Figure 11. Note head–trunk demarcation, external gills and suckers. 14, Magnified view of the head region of the experimental embryo from Figure 12. The neural tissue appears completely detached from the ectoderm and the neural cells are exposed. Note abnormal head shape and absence of external gills. 15, Dorsal view of the control embryo at 24 h. Note head–trunk demarcation. 16, Dorsal view of the experimental embryo at 24 h. There is no clear-cut demarcation of head and trunk region. Neural tissue is not covered over by ectoderm and there is a wide gap between neural cells and ectoderm. Neural folds appear to have fused at least partially. Scale bar = 100 μ m.

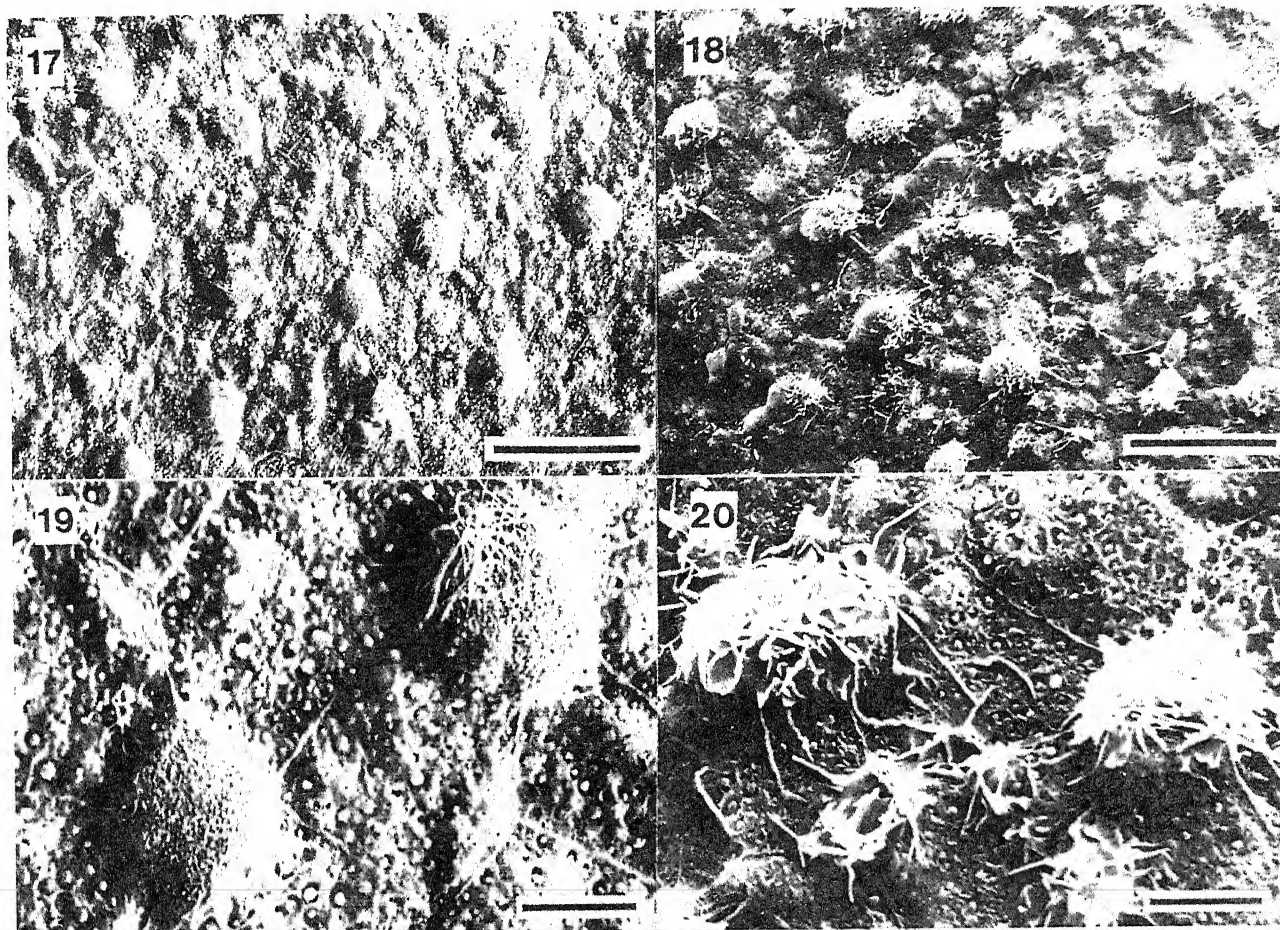
definite head, trunk and tail differentiation (Figure 11). The experimental embryos, however, showed considerably collapsed neural folds in the anterior two-third region of the neural tube. There was also overall incomplete differentiation of the head region while the tail was curved upwards (Figure 12). Slightly magnified views of these two figures are presented as Figures 13 and 14. Collapsed neural folds and protruding neural cells were clearly evident in the experimental embryos (Figure 14).

Dorsal view of the control and experimental embryos is presented in Figures 15 and 16, respectively. A considerable gap was observed between the neural and non-neural ectodermal cells of the experimental embryo. Nonneural ectoderm had apparently detached completely from the neural cells (Figure 16), a fact which was also evident from histological analysis (see Figures 6 and 8).

SEM examination of the surface epithelial cells of the control and experimental embryos revealed some striking changes caused by sea water exposure. The surface

of control embryo (trunk region) showed typical epithelial cells with surface projections and the usual ciliated cells arranged in a definite pattern (Figure 17). Corresponding region of the experimental embryo showed epithelial cells with abnormal surface projections. Ciliated cells were also abnormal (Figure 18). A more detailed view is presented in the Figures 19 and 20. Large 'ruffles' present on the cells from experimental embryo (Figure 20) were not at all observed in the controls.

The results presented herein clearly show that tidal inundation of the breeding pools will be detrimental to the embryos of *M. ornata*. Increased salinity of more than 30‰ concentration of sea water will either be fatal or it will cause abnormal development of the exposed embryos. Many aspects of salinity-caused abnormal development, such as prevention of swelling of perivitelline space, curved body axis, inhibition of hatching, etc. have been discussed earlier⁶ and need not be reiterated here. The purpose of the present paper is to discuss abnormal neurulation and related phenomena.



Figures 17–20. 17, Ectodermal cells on the trunk region of the control embryo. The typical pitted appearance and surface projections are apparent. Also note pattern of ciliated cells. Cell boundaries are the distinct white raised lines (see magnified view Figure 19). Scale bar = 50 μ m. 18, Markedly changed ectodermal cells of an experimental embryo. Note reduced ciliated cells and abnormal surface projections on many cells (magnified view Figure 20). Scale bar = 50 μ m. 19 and 20, Magnified views of Figures 17 and 18 respectively. Scale bar = 10 μ m.

Both processes, induction of neural tissue and morphogenesis of the neural tube, continue to attract the attention of research workers. A detailed review of recent advances in neural induction phenomena has recently been published⁹, while the mechanism of neurulation has been reviewed several times during the past 20 years or so by different workers^{10–12}. In addition, two excellent reviews of the experimental analysis of the shaping of the neural plate and tube are published by Jacobson^{13,14}. In spite of this, the actual nature of neural induction phenomenon and the mechanical as well as physiological aspects of neurulation are not fully understood.

As far as neurulation is concerned, there is still controversy regarding the forces involved in the rolling of neural folds to form the actual neural tube – whether the forces are entirely intrinsic (to the neural tissue) or extrinsic factors (nonneural ectoderm, mesoderm) also help the process¹². It is also becoming apparent now that

the mechanisms involved may be different along the antero-posterior axis of the neural tube^{12,14}. This may be the reason why only the anterior part of the nervous system was affected in our experiments. In fact, Burt¹⁵ also reported that neurulation was more retarded in the head region than in the spinal cord when the *Amblystoma* embryos were exposed to saline water. While studying the uptake of Ca^{++} in developing frog embryo, Barth and Barth¹⁶ also showed that cephalic regions incorporate more Ca^{++} than the spinal regions. In mouse, external Ca^{++} added in the medium was shown to augment the rate of neural tube closure in the hind brain region only and not in the other region, again pointing out that physiological mechanisms may be different at different places along the neuraxis¹⁷.

An interesting aspect of our finding is that the non-neural ectoderm got detached from the neural ectoderm, somewhere in the latter stages of neurulation, in the em-

bryos exposed to sea water. Apparently, in spite of detached ectoderm, neurulation continued, albeit abnormally. *Microhyla* embryos exposed to NaCl also show defective neurulation⁶, an observation in agreement with the present results. This is not surprising since the major cation in sea water is indeed sodium. It appears certain, therefore, that the concentration of cations/anions, especially that of sodium, in the water surrounding the embryos plays important role in morphogenesis. Exposure to cations like mercury and lead does not produce a similar effect in *Microhyla* embryos^{18,19}. The collapse of neural folds reported here is, however, very similar to that reported in rat embryos cultured in Ca⁺⁺-deficient medium²⁰. It may be worthwhile to investigate the effect of sea water/NaCl treatment on Ca⁺⁺ balance in *Microhyla* embryos and its relation with neurulation.

On the whole, the SEM structure of the control *Microhyla* neurula is similar to that described by Tarin²¹ and Löfberg²² for other amphibians. The surface ectoderm is showing all the features described by these authors. In experimental embryos, there were considerable cell surface alterations as a result of exposure to drastic change in the salinity. But the mechanism involved is unknown at present. Keller²³ has, however, noted that in *Xenopus* embryos high salinity (0.385% salt concentration) causes the epithelium to weaken and even break down. He further noted abnormal ingression of surface cells, thickening of blastula and gastrula walls as well as exogastrulation. However, we did not observe exogastrulation in the present study. This may be because we did not remove the vitelline membrane.

In conclusion, it can be stated that *Microhyla ornata* embryos will not tolerate excessive tidal inundation and the resultant increase in the salinity of their ambient pond water. The embryos are very sensitive to more than about 0.2% NaCl or 20% sea water and that high concentration of salt will cause abnormal development.

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The Many Faces of Science: An Introduction to Scientists, Values and Society. Leslie Stevenson and Henry Byerly. Westview Press, 5500, Central Avenue, Boulder, Colorado 80301-2847, USA. 257 pp. Price: US \$18.95. Hard bound US \$55.

The sociologist Paul Feyerabend's scathing dismissal of science – 'What's so great about it?' – is countered in this mildly diverting but otherwise forgettable book by two professional philosophers. Although it is not specifically intended to be an apology for science, the book does have a superficially feel-good flavour that scientists afflicted by self-doubts about their calling might take some comfort in. The lay reader is unlikely to be similarly soothed, but he or she will probably come away from the book with a number of morsels of information that could confound dinner companions, or win quizz competitions. It is interesting to read, for instance, that Count Rumford (he of cannon-boring fame) was a sort of Yankee upstart with a taste in rich widows who has given us, among other things, the sofa bed and the drip coffee-maker. Aficionados of trivia may enjoy learning that Niels Bohr, an early and important contributor to quantum mechanics, came to the attention of the US Secret Service when he worked on the atomic bomb project and was code-named Nicholas Baker. But they might also echo T. S. Eliot's doleful enquiry: After such knowledge, what forgiveness? because, in the end, Stevenson's and Byerly's book does not really rise much above the level of a compendium of facts and anecdotes, some amusing, some bland. There is a marked reluctance to confront issues (despite the promise of the title), and much care to be evenhanded in all things, whether from pusillanimity or from political correctness is not clear. The book is also marred by some inaccuracies, and the occasional howler. For instance, as evidence of Isaac Newton's willingness to take pains, the authors point to his calculation, to 55 decimal places (!) – of the area under a curve by series expansion. What must surely be meant is his use of 55 terms in the expansion. Einstein's 1905 paper on Brownian motion is said to have supplied the definitive arguments for the physical existence of molecules, an ob-

servation unlikely to have received a ringing endorsement even from Einstein himself. And chemists will be non-plussed to set Gibbs phase rule described as a method to determine the concentrations of various substances so as to get a desired mixture in which the components are in equilibrium and do not separate out.

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Symmetry Orbits. Hugo F. Verheyen. Birkhäuser Boston, 675 Massachusetts Avenue, Cambridge, MA 02139, USA. 1996. 236 pp. Price: DM138.

Texts on symmetry in the structure of objects used to be terse, abstract writings on a course of pure mathematics, even a few decades ago. The classic texts by Weyl and Hamermesh were beyond the reach of most physical scientists. There were hardly any illustrations. With the success of symmetry-based applications of quantum mechanics in understanding molecular structure and features of spectroscopy, a range of texts have appeared varying in their

audience level from an expert to a pedestrian. With greater access to computer graphics, more attractive illustrations were now expected.

It is in this aspect, namely the quality of illustrations, that the book *Symmetry Orbits* by Hugo F. Verheyen, comes out on top. It utilizes photographs of models, black and white computer graphics with excellent shading, as well as line drawings to help the reader in experiencing symmetry groups. The pictures are highly attractive and naturally demand attention. Unfortunately, having nearly 200 illustrations in a 234-page book imposes constraints on what else can be included in the text. There is an extensive classification of isometries and later, polyhedra, but in a language less familiar to a physical scientist. There are, perhaps for good reasons, no attempts to develop a theoretical framework to understand structural properties of objects like molecules. It seems that the author is more interested in involving the reader in the beauty and complexity of geometry than in discussing applications that follow from the occurrence of symmetry. To take an example, after reading this book, you can marvel at the beautiful design in the making of a fullerene molecule, that the molecular world indulges in; but to understand the role of symmetry in determining what internal modes will be accessible for interaction with the surroundings, one has to look for further texts.

Thus, the book is really meant for those who have a mathematician's interest in group theory. Thus, tetrahedral is A_4 and octahedral is S_4 . Of course, this does not reduce the value of the work, but it does limit the audience significantly. Unfortunately, the absence of an alphabetic index or a glossary makes it even more difficult for one more used to the Schoenflies symbols. The book is divided into two parts; the first with three chapters is titled 'Realization of Symmetry Groups' and the second with four chapters is called 'Compounds of Cubes'. Most of the text is on defining the terms and classifying various possibilities. The classification of the finite compounds of cubes is done in a very compact and yet exhaustive manner. The positions in an orientation with rotational freedom are worked out in detail and the angles are given to decimals of seconds.

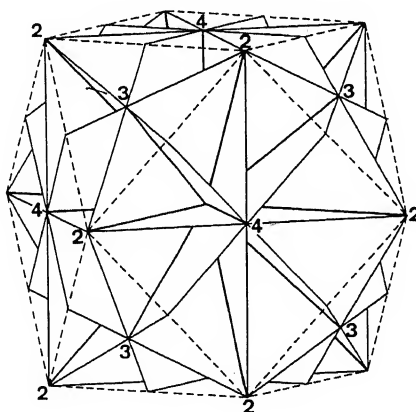
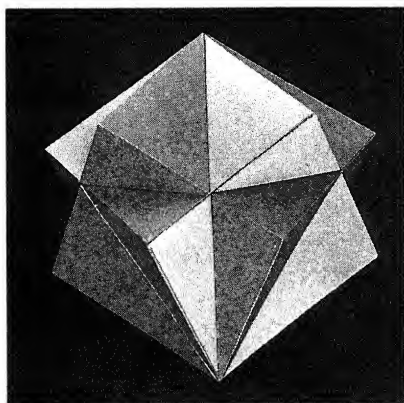


Figure 1. The symmetry operation of the complete octahedral group ($S_4 \times I$ or O_h ; order 48; E, I, 23 rotations, 9 reflections, 14 rotating inversions). Each operation is shown in terms of equivalent number of reflections in an imaginary cube. A rotation, for instance, is a product of 2 reflections.

a



b

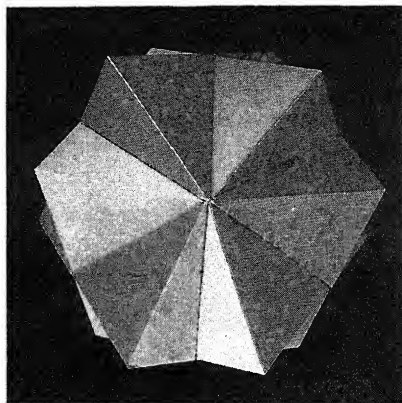


Figure 2a, b. Two positions of a $D_3 \times I$ group in its $C_3 \times I$ orientation, shown together as images of one another rotated through an angle of $22^\circ 14' 19''.52$. *a*, general view; *b*, along a threefold axis. A large number of such compounds (regular polyhedra with common centre) are introduced in the book for the first time.

Another perspective can come from artists or people who love activities like origami. In other words, the group theoretic insight can also mean for those

gifted in art, a rational outlook towards structured or not so structured design. To put it lightly, it could be interesting material for adults, who take toys seri-

ously. In chapter 7, titled 'Assembling Models', instructions are given on how to design 3-D models from cardboard pieces like a jigsaw puzzle. This is similar to constructing the Brillouin zones of a crystalline structure with the Miller planes clearly marked. This can indeed be a lot of fun.

There are a few things that should excite lovers of geometry. For instance, the photograph of a 2.3 metres high ball and stick model of platonic solids, that the author had made inspired by an old Dutch book written by H. Naber. The author's cat eventually brought this piece of art and mastery to extinction. A personal memoir of history leading to the publication of the book, in the appendix, is an interesting reading.

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Genetics and Evolution of Aquatic Organisms. A. R. Beanmont ed. Chapman and Hall, Cheriton House, North Way, Andover, Hampshire SP10 5BE, UK. 1994. 539 pp. Price: not known.

Genetics – survey, inventories and experimentation – of aquatic organisms is rather a slow starter. Emphasis in the early 70s used to be on the measurement of genetic variations; its relationship to environmental variability; its maintenance by selection and its use in taxonomy. However with the emerging field of biotechnology, the technical advances in the study of genetics and the increasing dominance of neutral theory as the explanation for genetic variation at both the protein and DNA level, a major shift is discernible.

Out of 44 contributions under segregated titles, 24 relate to marine species, 10 to limnetic domain and the remaining 8 to aquatic environment in general. Large abundance with patchy distribution is a hallmark of aquatic organisms, more specifically the marine species. However, the paradox is that most species do not have enough genetic variations. Consequently, the intraspecific genetic diversities having discrepancies

of very high magnitude, between abundance and population size still remain unexplained.

Another unanswered question relates to large temporal variations, particularly in marine biota. In spite of high gene flow, genetic differentiation on spatial scale requires differential survival of genotype after recruitment from planktonic larval stock. Of course, the large variance in reproductive process is mediated by hydrographic regimes. Thus there is a strong and direct linkage between population genetics and environment. Further, the large variance in reproductive process also affects population structure because of temporal and spatial variations in the genetic composition of the recruiting larvae. So far, the impact of DNA technology in population genetics is less than projected.

Talking about the evolution, several sets of biochemical databases (allozyme, immunological and DNA – RNA hybridization) have been applied to collate phylogenetic relationship among aquatic species. In terms of tools, the advent of polymerase chain reaction (PCR) technique has generated large amount of information on DNA, especially in fin-fishes. The population genetics studies

on a wide range of agametic clones provide information on the evolutionary consequences of the basic differences between agametic clones in terms of genetic variability and extent of ecological differentiation. In this context, an unanswered question in chromosome genetics is 'do marine species frequently exhibit greater number of chromosomes than freshwater species?'

Genetic research complimentary to pollution monitoring programmes is becoming indispensable. Pollution-mediated mutations directly cause damage to the DNA molecule within the individual cell nucleus, resulting in gene mutation or chromosomal aberrations, though not much is on record about chromosomal aberrations. Another discernible influence of pollution is through selective pressure on the genetic structure of the population by modifying the environment. Heterozygote genotypes, because of their less energy demanding maintenance have better chances of survival in stressful environment.

Aquaculture has tremendous scope for genetic research, especially the quantitative genetics; chromosome ploidy manipulation; allozyme genetic and transgenic organisms. An important

observation is 'standard errors on heritability estimates are uncomfortably larger unless.... based on very large numbers'. Hybrids which are heterotic for growth and resistant to disease have to be the goal as evidence from the success of different strains of crabs and catfishes indicates. In spite of the inherent sterility of triploids, a promising approach is to induce tetraploidy by cell fusion. Rather a disheartening finding for fish farmers is the repeatedly significant loss of genetic diversity following hatchery culture. Studies on

effects of cultured fish on wild populations have demonstrated that in many cases, coadapted gene complexes may be broken down and unique alleles lost in wild population, following extensive hybridization and introgression of genes from cultured organisms. Transgenic involving DNA techniques is currently centered around transfection, i.e., the introduction of novel genes in aquaculture species. The number of genes which might be appropriate for genetic engineering in finfishes and several growth genes have now been identified and cloned.

The contents are mainly confined to reports from Europe. It warrants an early compilation of results from the rest of the world, so as to have a global perspective.

Indeed a good read.

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Some earthquakes of Kashmir from historical sources

R. N. Iyengar and Devendra Sharma

Collection of information on past earthquakes is essential for proper assessment of the seismicity of a region. Even though the Himalayan continental collision zone is known to be highly seismic, data on earthquakes prior to the eighteenth century are scarce. This may be contrasted with the Eurasian continental boundary zone, wherein past data have been pieced together¹ to go as far back as second century A.D. In recent years due to the increasing human intervention in the river valleys, there has been a heightened sensitivity towards seismic events occurring in the Himalayan region. A question of considerable engineering and scientific significance is the return period of large earthquakes in the Himalayas. Recently Athavale² has highlighted the use of historical records in improving our database on earthquakes in India. We attempt to bring here to the attention of the readers that reliable information is available about a few earthquakes in Kashmir.

History of kings of Kashmir³

Among the innumerable books that were written in Sanskrit language in medieval India, *Rajatarangini* occupies the pride of place as the only one which can be classified under History. The author of *Rajatarangini* is the famous Kalhana who lived in 12th century A.D. The author has attempted the genealogy of the kings of Kashmir starting from very early periods (2448 B.C.) up to 1148 A.D. Kalhana was a contemporary of king Sussala (1112–1127 A.D.) and king Sinhadeva who ascended the throne in 1127 A.D. Kalhana's narration has been continued by Jonaraja as *Rajavali* up to 1412 A.D. Srivara Pandit, a disciple of Jonaraja further covered the period up to 1477 A.D. The last and further part of the history is by Prajya Bhatta who has written *Rajavali Pitaka*. This gives the history of Kashmir up to 1587 A.D. when Akbar conquered the kingdom of Kashmir. All the above books have been translated into English by J. C. Dutt³.

A total of four earthquakes find mention in the history of Kashmir. The details are as follows.

Earthquakes in 12th century A.D.

Kalhana describes the reign of Sussala in great detail. Sussala lost the kingdom for a brief time and regained the same from Bhikshacara (Bhikshu). During his second reign, Sussala had to put down many rebellions and enemies. While the king's soldiers were engaged in battles with the followers of Bhikshu on the banks of river Kshiptika, Kalhana writes³: 'The Sun became fierce, there were earthquakes several times, and terrible storms blew breaking down many trees. The dust raised by the storms seemed like pillars raised to support the sky which was rent by blows.' The description is more graphic about the storm than about the earthquakes. However, Kalhana wrote this part of history pertaining to Sussala from personal knowledge and thus the occurrence of the above earthquakes near Srinagar (capital of the King) during 1121–1125 A.D. may be reliable.

Major earthquake in September–October 1554 A.D.

The most detailed description of an earthquake which occurred in the reign of king Shamsha Shah (1537–1559 A.D.) has been provided by Prajya Bhatta. The translation of Dutt³ is reproduced here: 'In the month of Ashvina of the year 30 there occurred frequent earthquakes on account of the wicked acts of the king, as if the earth suffered from flatulency. The planet which causes calamity is assuaged by various acts, by gifts of land to independent people, by giving back to men their properties which had been robbed, and by like deeds. Now there occurred an earthquake at the second watch of night when all men were asleep, and it destroyed many people. It caused holes

in the ground, and travellers going on their way were misled at every step. Houses fell into these holes at night and the people, anxious to get out from their houses in the morning, issued by breaking through the roof. On this occasion many wooden houses fell into the water of the Vitasta and when they had floated down for seven kroshas, the people who were in them awoke and came out. The confusion, caused by the earthquake in the towns of Hasainpura and Hosainpura, situated at some distance across the river, can be seen even to this day. Pitiabie cries of lamentation of the much afflicted people were then heard calling out "O father", "O mother!", "O friend", "O brother!" in different places, which made the heart feel as if it were struck by a thunderbolt. At this time the sky appeared terrible with the claps of thunder, the movements of the stars were stopped, and the land was agitated like a gourd on the waves. The mind of the people became troubled with the fear of the earthquake, and they felt no affection for sons or friends or wives or for good men or for kind-hearted people or for any object whatever. It was owing to the glory of the holy shrines of Vijayeshvara, Martanda, and Varahakshetra, that fears and apprehension from earthquake were not felt by the inhabitants of these places. The earthquake continued for several days, occurring several times every day, and all the people lived under canvas.'

Two minor shocks

Two earthquakes are passingly mentioned in the text. Both refer to the sixteenth century. The first was during the reign of Gazishah who ruled Kashmir for two years in 1560–1561 A.D. The text reads, 'The king saw conflagrations in different directions and there occurred earthquakes and so he took council of soothsayers....'.

The next event which from its description appears to be an earthquake has been described during the reign of Ali Shah (1569–1577 A.D.). The narra-

tion is as follows: 'But suddenly the sky became red on all sides, like the fierce fire that will appear at the end of the world; it prognosticated destruction by famine. The world showed symptoms of a calamity and trembled, as if unable to bear the weight of a famine.'

The famine is further described in detail. However the trembling of the earth mentioned may not be just figurative.

Conclusion

Search of historical literature and records gives valuable information regarding regional seismicity and may even help scientists in refining recurrence intervals of seismic activity. There are innumerable references and speculations about earthquakes in ancient and medieval Indian writings. But information on the dates and location of actual

shocks is not available. Thus, the description, provided by the historians of Kashmir, of the shock of 1554 A.D. is perhaps unique.

The Cambridge history of India in the chapter 'Kingdom of Kashmir' mentions a great earthquake which changed the course of river Jhelum. This is perhaps the same as the one described previously. Also this may be same as the 1552 A.D. Srinagar event listed in the earthquake catalogues. The description of the shaking: 'It caused holes in the ground and the land was agitated like a gourd on the waves The earthquake continued for several days, occurring several times every day.' indicates severe shaking with a main event of MMI intensity IX-X and Richter's magnitude reaching 7 or higher values. Cross references from other literary sources may throw more light on the felt area of the earthquake. All the places

mentioned in the text are identifiable. For example Varahakshetra or Varahmula, is the present day Baramulla. Hence palaeoseismic studies in the Kashmir valley will also prove to be valuable.

1. 'Catalogue of earthquake in Armenia' in *The Armenia Earthquake of December 1988* (eds Yegian, M. K. and Ghahraman, V. G.), N. E. University, Boston, USA, 1992.
2. Athavale, R. N., *Curr. Sci.*, 1995, **69**, 279-280.
3. Dutt, J. C., *Kings of Kashmira*, Mittal Publishers, New Delhi, India, 1887 (reprinted 1990), vols I-III.

ACKNOWLEDGEMENT. We thank DST for financial support.

R. N. Iyengar and Devendra Sharma are in the Central Building Research Institute, Roorkee 247 667, India.

Erratum

Metallization of hydrogen – Everest conquered?

[*Curr. Sci.*, 1996, **70**, 876-877]

S. Ramaseshan

Read '140 GPa' instead of '140 megabars' in 1 column, 8th line on page 877.

MEETINGS/SYMPOSIA/SEMINARS

20th International Congress of History of Science

Date: July 1997

Place: Liege, Belgium

The Indian National Science Academy plans to present at the Congress a Status Report on Studies in History of Science in India, covering the period 1993-1997. The National Committee for International Union of History and Philosophy of Science (IUHPS) requests individual researchers and institutions to finish brief reports on studies carried out in the field of history of science, from April 1993 to date. The report should be very concise and in third person so that an author's work is preferably reported in his own words. Offprints of papers on which the report is based as well as a list of publications for the period should also be sent. The institutions may like to send a consolidated report. They should provide the following information: 1. Book/monographs published; 2. Symposia, conferences, schools, etc. organized; 3. Library and archival services available; 4. Complete postal and e-mail address along with phone and fax numbers (including area code) for compilation of a directory. Material should reach before 30 September 1996 to:

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2. Constantine, G., in *Biology of Bats* (ed. Wimsatt, W. A.), Academic Press, New York, 1970, vol. 1, pp. 319-322.

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INDIAN COUNCIL OF AGRICULTURAL RESEARCH

The Indian Council of Agricultural Research, New Delhi announces the following existing and new awards:

Best Institution Award, 1996

In order to recognize the best performance in Agricultural Research and Education, three ICAR Awards of rupees one lakh each will be given to two ICAR Institute/N.R.C./Project Directorate and one state Agricultural University, partially out of the interest accrued from the King Baudouin Award bestowed on the ICAR in 1989. Those institutions which have received the award earlier in 1995 need not apply.

Vasant Rao Naik Award, 1996

All scientists or extension workers who have made outstanding contribution in the areas of Water Conservation and Dryland Farming in India shall be eligible for the award. One award of Rs 100,000 is to be given annually, for the work done during the preceding five years of the award.

Outstanding Women Agricultural Scientist Awards, 1996

All the women scientists working in the Institutions under the ICAR system including State Agricultural Universities are eligible for the award, one award of Rs 25,000 is to be given away annually for the significant contributions made during her career as an agricultural scientist.

Hari Om Ashram Trust Awards, 1995-96

All scientists engaged in research in the field of Crop Sciences, Horticulture, Resource Management and Animal Sciences in India shall be eligible for the award. Four awards of the value of Rs 20,000 each are to be given once in two years, for the work done during the preceding five years of the period of the award.

ICAR Young Scientist Award for Agricultural Research, 1996-97

All young Scientists/Lecturers/Assistant Professors and who have obtained the Ph D degree and are below the age of 35 years shall be eligible for the Award which will consist of the sanction of a really challenging scheme to be submitted by the candidate and a cash prize of Rs 10,000, 50 per cent of which will be given at the time of the award of research grant and the remaining 50 per cent will be released on the successful outcome of the scheme. A total of 10 awards will be made in various disciplines of agricultural and allied sciences once in two years.

ICAR Awards for Team Research, 1994-96

All the persons engaged in research in the field of agriculture animal husbandry and fisheries sciences in India shall be eligible for the award. Eight awards of Rs 50,000 each are to be given once in three years for the work done during the preceding three years of the period of the award.

Dr Rajendra Awards, 1994-96

The award is open to Indian authors including Editors of multi-author book where the Editor has himself contributed substantially together with an editorial preface. Both published works and manuscripts proposed to be published by its author will be accepted provided that such a work is written originally in Hindi and does not infringe the copyright of any other person. Eight Awards of Rs 20,000 each are to be given once in three years for the work done during the preceding one year of the period of the award.

The application form for the above awards may be obtained from **Dr R. C. Maheshwari, Assistant Director General (CSC), ICAR, Krishi Bhawan, Dr Rajendra Prasad Road, New Delhi 110 001 on or before 31 October 1996** by sending a self-addressed two-rupee stamped envelope. Six copies of the application may be forwarded by the Head of the Institution/Directors of Institutes/State Department of Agriculture/Horticulture/Animal Science/Fisheries and Vice-Chancellors of State Agricultural Universities. **The last date for submission of applications for these awards is 16 December 1996.** The last date for candidates in the Andaman and Nicobar Islands, Lakshadweep States, Union Territory in the North Eastern Region, Ladakh Division of J&K State and Sikkim is 31 December 1996.

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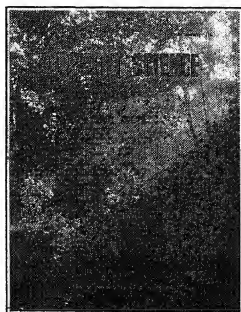
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COVER. A view of the mid-elevation wet evergreen forest of *Cullenia-Aglaiia-Palaquium* at Kalakad-Mundanthurai Tiger Reserve, Agasthyamalai range. See page 379.

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In this issue

Gene therapy

Curing diseases or playing God? Luddites often wrongly frame the question in this manner. Genetic manipulation of organisms, using recombinant DNA methods, is now commonplace. Rapid advances in technology and in our understanding of the mechanisms underlying many diseases allow us to realistically consider methods which alter human DNA to treat illnesses. Should we permit such approaches to medical treatment? Is it safe? What are the ethical implications? Are we playing with Nature when we alter an organism's DNA? Will we create a monster we cannot handle? There is only one answer to all these rather complicated questions: The application of recombinant DNA techniques to medicine, farming, industry, etc. does indeed raise important ethical, social and legal issues. The extent to which answers are known, the implications and the issues all vary from case to case and even with the social context. General answers are not only not possible, particular answers will change rapidly with time. In this situation, the only solution is for biologists to be in constant open communication with their societies so that an ongoing discussion with doctors, lawyers, social leaders, politicians and the lay public is always maintained. Decisions about what is possible and desirable should therefore be based on informed consensus. The credibility and openness of the scientific community is essential if medical science is to benefit from the immense possibilities becoming available.

The situation in many societies, including ours, is unfortunately quite the opposite. Post-World War II euphoria about science as the vehicle for eradication of poverty has

given way to suspicion of technology. We are often asked to choose between economic development and the rape of the environment as if one must live in tribal splendour, at one with Mother Earth, or face the wrath of the Gods for daring to alter Nature. As a society, we must accept informed debate as a method for reaching agreement and as scientists we must place technical facts in an accessible form and appreciate concerns of social and cultural origins without being dismissive of them.

Let us take specific examples. Duchenne's muscular dystrophy is a sex-linked disease found in India and in the rest of the world. The gene responsible for the disease has been identified and studied in great detail. It is possible to rather easily diagnose if a developing foetus is male and carries the mutant gene. The human who develops with this defect will surely die early in his youth and will suffer greatly. If it were possible to inject his muscle cells with targeted DNA encoding the normal gene product, so that he is 'cured' and leads a relatively normal life, is it ethical? After all, we give thalassemics blood transfusions, can we not inject into the developing foetus genes which allow them to make normal blood, but do not affect them in any other way? Many biologists will give a qualified yes to these questions, their views depending on the exact method proposed. Many religious and social leaders may object, on the ground that one should not interfere with a developing foetus.

A critical requirement for informed debate on these questions is the availability of facts to discuss potential risks and possibilities. Facts alone are not enough to convince our societies about specific scientific agendas, but facts are es-

sential. And facts on the possibilities can be available only by experiments on model organisms. Gene replacement is possible and has been done effectively in bacteria and yeast and is possible in animals such as mouse. Foreign genes have been introduced in a variety of organisms ranging from bacteria, plants and animals. These studies have allowed a rational analysis of specific possibilities of gene therapy in human. However, much needs to be learnt about how DNA-based therapy can be performed in human. What are the vehicles of delivery of the DNA? How will the DNA be targeted? How will it express in the required amount in the correct tissue and developmental stage? Rangarajan and Padmanaban (page 360) address these questions by reviewing rodent models for gene therapy and comparing them to what is possible in human. They present an analysis of their experimental approaches and possibilities. Comments on their report and similar reports from people studying other aspects of medical applications of modern biology should allow discussions to begin on an important issue.

K. VijayRaghavan

Soaps and history

Every child (and adult) who has blown soap bubbles will bear testimony to the marvellous properties of surfactants (a name derived from the term *surface active agents*). From soaps and detergents to emulsifiers and foaming agents, surface active molecules find myriad applications, that permeate almost every aspect of daily life. In biology, the wonderfully fashioned architecture of cell and organelle membranes relies on the lipid bilayer; a superb example of self-organization driven

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ther stated that 'leaving the work of the institutions in the hands of older scientists may reduce the overall viability of Indian research'.

In any organization, if old age has hindered or adversely affected the innovativeness and or creativity, then to uphold discipline among the scientific community, the policy of promotion or recruitment has to be suitably amended. Further, the performance and contribu-

tions of scientists, irrespective of their positions are to be rigidly and periodically reviewed.

However, in this regard, it is imperative to remind ourselves that the *present young* will be the *future old*. A scientist whether young or old should be in quest of truth and knowledge. In various disciplines, many of the theories and hypotheses, put forth by the earlier scientists have even to this day remained as the basis for any

future studies. Let us salute the profound knowledge and insight of the old guardians of science!

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NEWS

Uncommon opportunities for a food secure world

There can be nothing more urgent than ensuring a food-and-nutrition-secure world where all of humanity will have enough to eat and be free from malnutrition now and in the future. This, however, is a formidable task. Attempts made in the past have not yielded the desired results. The situation continues to be one of deep concern. It is to tackle this problem that the FAO, which has been continuously addressing the question of feeding the world for the past more than 50 years, is convening the world food summit, a high level meeting of Heads of State and Heads of Government, in Rome during 13-17 November 1996.

Many of our resources - aquifers and the ozone cover, for example - are being depleted unsustainably. The natural-biological resource base has steadily deteriorated. Loss of biodiversity, soil erosion and desertification have gradually reduced the productive capacity of agricultural land. In some cases, this process has become irreversible, pointed out Elizabeth Dowdeswell, Executive Director, UNEP. 'The earth's capacity to produce enough food to satisfy our expanding demand is now emerging as the overriding environmental issue as the world approaches the 21st century', says Lester Brown of the World Watch Institute. And 'food security' is just one, albeit the major, component in the ongoing population-resource debate. There are other factors such as unprecedented demand for energy and other resources, quality of life, technological options, political freedoms, economic arrangements,

notions of justice and equity, and living in peace with other creatures with whom we share the planet.

Thus the tasks confronting humanity today are mammoth and complex and call for concerted effort of people from different streams of life. Surely, science has to play a crucial and central role if the desired goals are to be achieved. It is with this conviction that several Science Academies of the world decided to pool their collective wisdom to address the major issues of food and nutrition security and prepare a work agenda to be placed before the November Summit of political leaders.

The initiative came from the National Academy of Agricultural Sciences of India, Accademia Nazionale Delle Scienze, Rome, and the Third World Academy of Sciences, Trieste, to organize a four-day Science Academies Summit to reflect as scientists on the theme of 'sustainable food security' and to delineate the role of science and scientists in ensuring a balanced food basket for an ever-increasing population. This meeting was supported by Food and Agriculture Organization (FAO), Ministry of Foreign Affairs, Government of Italy, United Nations Development Programme (UNDP), and the Encyclopaedia of Life Support Systems (EOLSS).

At the Science Summit convened at the M.S. Swaminathan Research Foundation, Madras, during July 8-11, 1996, there were representatives of fifteen Science Academies including the Caribbean and Chinese Academies as well as the

Academies of Kenya, Uganda, Hungary, Lithuania and Pakistan, and international organizations including FAO, UNDP, UNEP, UNESCO, and CGIAR. The meeting was addressed by, among others, Gaetano Zucconi, Italian Ambassador to India, Ministers of the Government of India Chaturanan Mishra and Yogendra Alagh, elder statesman C. Subramaniam, and Tamil Nadu's Minister for Agriculture Veerapandi Arumugam.

The summit commenced with welcome addresses delivered by M. S. Swaminathan, President of the National Academy of Agricultural Sciences of India, G. T. Scarascia Mugnozza, President of the National Academy of Sciences of Italy, Johanna Dobereiner of the Third World Academy of Sciences, Peter Rosenegger, FAO Representative for India and Bhutan, and Hans-C. von Sponeck, Resident Representative of UNDP in India.

Y. K. Alagh, Minister for Planning and Science and Technology, reiterated the need for comprehensive measures that would ensure long term sustainability and said short-run crisis management would only lead to serious adverse consequences. Recognizing the key role of information dissemination, he emphasized the need to adapt the extension system for it to be able to convey the tremendous technological developments that are taking place now. He would also like to have in place a good system of patenting indigenous innovations that would make them freely available to the local people. For his part, the Union Minister for Agriculture,

Mishra, pleased the scientists assembled telling them that governments should invest a high percentage of their GDP on agricultural research. For example, he would like India to invest at least 1.0% as against 0.3% of GDP being spent now. Another suggestion he made was to have a system of social audit of research institutions.

While the Minister's ideas on government support are surely welcome, Elizabeth Dowdeswell felt that we must not depend upon the government for everything and we should also win the support of the private sector. There can be no talk of sustainability until the disparities in patterns of economic growth and consumption have narrowed. Women, in particular, have been marginalized by resettlement schemes and land tenure policies that emphasize male households. There should be a gender assessment study of each project going on farm, said Dowdeswell.

Food security is not a static concept and it changes with new life styles and new human habits. So do the tools to achieve it, pointed out Adnan Badran, Deputy Director General, UNESCO. Food security, thanks to the ongoing revolution in genetic engineering, will soon be determined on the lab-bench by interdisciplinary programmes yielding new strains resistant to diseases and insects and high protein diets from organic compounds produced biologically from algae, fungi, bacteria or synthetic hydrocarbons. Badran drew attention to the tremendous North-South disparities in the number of scientists and in the expenditure on Research and Development. He highlighted the different ways scientific research can help achieve food security, such as molecular biological research on agronomic, horticultural plants and animal husbandry to increase the yield, and R&D in genetic engineering for (a) high yield strains, (b) new crops, (c) resistant varieties to disease and insects, (d) dwarf varieties for intensive crop production, (e) post harvest longevity, (f) augmenting quality to consumer taste, and (g) adaptability to stress physiology.

Iba Kone strongly felt that the use or transformation of DNA strains for any purpose other than the benefit of humanity should be outlawed by the International Court of Justice at The Hague. Ismail Serageldin, Chairman, CGIAR, was concerned that the tremendous North-South

knowledge gap could lead to a new class of scientific apartheid.

Johanna Dobereiner emphasized the urgent need to establish regional centres of excellence dedicated to high quality training and research in critical fields such as food crops, agroforestry, agronomy, agrometeorology, soil and water management, and pest control in ecologically complex and fragile regions, and in the fields of molecular biology, genetic engineering, biotechnology and informatics. In her view, the success of India's Green Revolution has clearly established agricultural research as a major contributing factor in enhancing food security. She pleaded for greater North-South cooperation in research as well as fruitful industry-research-farmer interactions. It is important, she said, to develop fuel from bio-mass as has been done in Brazil and to select appropriate high-yielding varieties that would not need high doses of fertilizers.

R. S. Paroda, Secretary, Department of Agricultural Research and Education, Government of India, highlighted the tasks involved in Eco-regional Research and Development, such as identifying and prioritizing research issues common to different ecoregions and developing mechanisms for collaborative programmes involving National and International Agricultural Research Systems, FAO, UNDP, donors and NGOs as well as monitoring the improvements in the eco-region according to predetermined sustainability criteria. On the management issue, he emphasized the need for research on (i) the conservation and management of eco-systems that include multi-crop integrated farming systems in a programme mode, (ii) the management of production systems, and (iii) factors affecting farmers' incentives and adoption of improved technologies.

Jyoti Parekh, from Indira Gandhi Institute of Development Research, spoke on the importance of developing environmental auditing approaches and constructing environmental indicators. She suggested that environmentally sound farm management practices should be rewarded.

B. K. Sinha opined that food technology should play a greater role in popularizing coarse grains and root and tuber crops. Clement Sankat also thought that there is a need for us to shift our focus from cereal grains, rice, wheat and maize to

less exploited crops like roots and tubers, pulses (pigeon pea), oilseeds, fruits (Sapodilla, Cassava and Carambola), herbs and spices (Coriander). Self-sufficiency of individual countries or by a group of nations in a region with a consideration of climatic conditions may provide a stronger basis for food security of developing countries.

Obaidullah Khan of FAO could not agree any less with Y. K. Alagh in the need for winning recognition for the concept of community knowledge which is currently being threatened by the IPR regime. An underlying hard political issue of rural societies is the participatory control of and access to natural resources by those who are dependent on those resources. Another area of concern is the inadequate use made of the distinctive knowledge of women as far as food security and biodiversity are concerned.

Dominic Makawiti of the Kenyan National Academy of Sciences would like traditional foods made of highly nutritious and drought-resistant millet, sorghum and cassava to be revived and popularized through education. Hotels and restaurants, he said, should present traditional foods in a modern way that would make them more palatable. He would also like the public to be made guardians of the local environment.

An overview of what modern science can do to the improvement of post production systems was provided by Joseph Hulse. The possibility of using mathematical models and computer technologies in determining efficient means of placing and integrating all the components essential to safe and efficient preservation, transformation, distribution and marketing from rural and maritime producers and harvesters to urban markets and consumers is indeed attractive. He would like the National Agricultural Research Systems to be strengthened and the CGIAR Centres to pursue a policy of subcontracting many of their present responsibilities and activities to NARS and regional research networks.

While most of the Science Summit was concerned about what science and technology could do, Amir Muhammed, President, Pakistan Academy of Sciences, went beyond science and technology and emphasized the need to bring in management techniques to agriculture, to involve social scientists and to give highest priority to land reforms, streamlining land records, land consolidation and land tenure.

The Science Summit brought into sharp focus the urgent need to address the issue of food security with all the knowledge and skills at our command. The meeting threw up several cases from actual successful experiences worldwide and emphasized the need to blend the traditional agricultural practices with what modern biotechnology has to offer. What we need, probably, is a people-oriented

Super Green Revolution that can ensure all the benefits of the Green Revolution on a global scale minus its environmentally harmful effects, and at the same time make economic sense. We need a research agenda that integrates sustainable development, food security and the environmental concerns.

The mood is one of cautious optimism. While the tasks facing us look daunting,

it is felt that one can win the battle with an appropriate blend of scientific and technological inputs and conducive public policies.

Ruchi Bhandari and Subbiah Arunachalam, M.S. Swaminathan Research Foundation, Madras 600 113, India.

Madras Declaration of the Science Academies Summit

The text of the declaration adopted at the Science Academies Summit convened at the M.S. Swaminathan Research Foundation, Madras, during 8-11 July 1996, is reproduced below.

We, the participants of the Science Academies Summit, call upon the world leaders assembling at the World Food Summit convened by FAO in Rome in November 1996 to adopt this agenda as a means of harnessing science and technology for the transformation of agriculture into a primary instrument of a global *Evergreen Revolution*. Based on the principles of ecology, social equity, energy efficiency, employment generation and economic viability, this revolution will provide the technical foundation for the universal eradication of hunger and achievement of a food and nutrition secure world for all. At the same time, we wish to emphasize the urgent need for adaption, particularly by developing countries, of population policies which can ensure that children are born for happiness and not for mere existence.

A new revolution in agriculture

In the over 50 years since FAO took up the challenge of 'food for all', never before has science offered greater opportunity to achieve this goal for even the poorest of the poor. Scale-neutral innovations including those emerging from the fields of biotechnology and information technology, as well as the holistic management systems of soil health care, conjunctive water use, integrated pest management and integrated intensive farming systems represent only a few of the new opportunities to reach the nearly 800 million people lacking adequate nutrition.

Tapping this unprecedented potential will depend upon strengthening the capacity of national agricultural research and development systems to respond to

these new challenges with creativity. Therefore, we urge world leaders to reverse the global trend of disinvestment in agricultural research and development, convinced that such short-sighted policy can but only have tragic results. At the same time, limited resources make prioritization of research initiatives essential, and it is intended that this agenda assist political leaders in that task.

Meeting the challenge of increasing food availability now and in the future demands equal focus on production systems and on the larger issues of access to food. Therefore, science must work in partnership with farmers to create a new agriculture. An Evergreen Revolution must bridge the gap between the past's gains in production and the persistent need for reliable access to food by all. This will require a number of innovative approaches, including:

- Transformation of the most marginalized farmers of the world into agents of poverty alleviation and environmental management through the blending of traditional and frontier technologies in socially equitable, economically viable and environmentally sustainable *ecotechnologies*.
- Production of more food from a diminishing resource base, requiring new agricultural technologies and management systems providing *increased productivity* per unit of land, water, energy, labour and investment. Part of this will involve focussing research on neglected crops such as minor millets, grain legumes and tubers which can perform in times of environmental stress and in neglected areas such as arid and semi-arid coastal and mountain areas.

- A *systems approach* marshalling the combined and coordinated efforts of physical scientists and agricultural researchers, as well as systems analysts, mathematicians and social scientists. While agricultural production will remain the foundation of food and nutrition security, the larger scientific framework must integrate post-harvest technology, distribution systems and rural development, as well as economic and social empowerment of the poor, especially women. This holistic approach must also be taken in restructuring administrative systems, leading to a high degree of professionalism of such services.

Eliminating hunger among the poor of all nations will depend upon tapping the new opportunities offered in these *unconventional approaches*. Such *uncommon opportunities* are rooted in a new and broader conception of food and nutrition security which integrates multiple physical, social, economic and environmental dimensions.

National policies for sustainable food and nutrition security should ensure

- that every individual has the *physical, economic, social and environmental access* to balanced diet that includes the necessary macro- and micro-nutrients, safe drinking water, sanitation, environmental hygiene, primary health care and education so as to lead a healthy and productive life.
- that food originates from efficient and *environmentally benign production technologies* that conserve and enhance the natural resource base of crops, animal

husbandry, forestry, inland and marine fisheries.

The principle operational implications of the above mission statement are the following:

1. The *physical dimensions* of food and nutrition security will involve a transition from chemical- and machinery-intensive to knowledge- and labour-intensive farming technologies.
2. The *economic dimensions* of food and nutrition security require the promotion of sustainable livelihoods through multiple income-earning opportunities, such as crop-livestock-fish integration and agro-processing and agri-business.
3. The *social dimensions* of food and nutrition require addressing gender, class and ethnic discrimination against marginalized sectors of society, who consequently tend to be the most food and nutritionally insecure.
4. The *environmental dimensions* of food and nutrition security involve attention to soil health care, water harvesting management and the conservation of biodiversity, as well as to sanitation, environmental hygiene, primary health care and education.

Ultimately, self-reliance and skill- and labour-intensive technology must be the basis of food and nutrition security. As agriculture provides most of the jobs in many developing countries, the import of food by these nations would be equivalent to importing unemployment.

A ten point scientific and public policy agenda for sustainable food and nutrition security

The following ten-point agenda can provide the basic scientific and policy framework for achieving sustainable food and nutrition security at both national and international levels.

1. *An Evergreen Revolution must increase output in an economically viable, socially equitable and environmentally sustainable manner*, focusing on the food and nutritional supply system as a whole. Beyond investing in new scientific technologies, this will require public policies which provide a supportive economic and social environment.
2. *Science and technology for public good is the key to improving agricultural productivity among the poor*. With the spread

of free-market and intellectual property rights culture, it is essential that science designed for the public good receives adequate political and financial support. Scientists working in the areas of food and health security should regard themselves as trustees of their intellectual property.

3. *Sound environmental policies must provide the foundation of agricultural sustainability*. Therefore, a national Natural Resources Conservation and Enhancement Strategy will be fundamental to a National Food Security System. High priority must go to combating desertification and deforestation, and to restoring degraded land.

4. *Entitlements, asset reform and technological empowerment of the poor will be essential in ensuring economic access to balanced diets*, and would help address the triple goals of natural resources conservation, poverty alleviation and food security.

5. *The gender perspective must be integrated into technological development and dissemination*. A gender code, to identify and evaluate actions that ensure equity in food and nutrition security, should become an integral part of agricultural research programmes.

6. *Agriculture must serve as an instrument of income and livelihood opportunity as well as of food production*. Therefore, it is important that the economic benefits of agroprocessing and agribusiness are taken to poor families through rural value added enterprises and partnerships with the private sector.

7. *Macro-economic policies in the areas of pricing, trade and investment should be based on both environmental sustainability, as well as gender and social equity*. A systems approach must be taken, with a holistic view of production, distribution and consumption.

8. *The information age has provided tools such as the Internet and GIS mapping to promote a learning revolution in agriculture*. Extension information should be disseminated through computer-aided information shops operated by village youth. Vocational polytechnic institutes may be established for the rural poor.

9. *Existing global conventions must be implemented*, including those on climate, biodiversity, desertification and the oceans, as well as Agenda 21 of UNCED and the global plans of action on population, gender, habitats, social development and plant genetic resources.

10. *Public policies for sustainable food and nutrition security must institutionalize procedures to focus on both production and access*. To achieve the above, it will be prudent to develop legislation based on the following principles:

A. *National Sustainable Food and Livelihood Security Act*, including provisions for:

- Promoting policies which can help to achieve a balance between human and animal populations and the supporting capacity of the eco-system.
- Promoting conservation and enhancement of the natural resource base.
- Rehabilitating degraded soil, forests and aquatic resources, and introducing scientific land and water use policies.
- Ensuring economic and social access to food through steps which can enhance the livelihood security of the rural and urban poor, with special attention being given to children, orphans and women.
- Improving the biological absorption and retention of food through attention to sanitation, environmental hygiene and primary health care.
- Ensuring universal literacy and technicity (i.e. imparting new technical skills through learning by doing) for both men and women at the village level.
- Promoting the development and dissemination of ecotechnologies at the production and post-harvest phases of farming, with special attention to waste treatment and recycling.
- Improving post-harvest technology including storage, non-CFC-based refrigeration, packing with biodegradable material and efficient transportation and delivery.
- Establishing input and output pricing and credit and insurance policies which can help all farm families, irrespective of their innate input-mobilizing and risk-taking capacity, to benefit from new technologies and marketing opportunities.
- Building and maintaining grain reserves and operating an efficient public distribution system for making essential commodities available at affordable prices to the poor.
- Developing a *Hunger-Free Area Programme* in cooperation with local communities in order to demonstrate that chronic hunger and malnutrition can be overcome speedily by creating an enabling environment, where every individual earns his or her daily bread.

B. Implementing the equity provisions of the Biodiversity Convention

Industrialized nations should contribute 0.01% additional ODA for the purpose of being credited to a Global Fund for Biodiversity for Sustainable Food Security. Such a fund can be handled as a trust fund under the Global Environment Facility (GEF) for implementing the Global Plan of Action adopted at the International Technical Conference on Plant Genetic Resources held at Leipzig in June, 1996, and for recognizing and rewarding the contributions of indigenous and rural women and men to the conservation and enhancement of biodiversity, that is, Farmers' Rights. It should also be used to safeguard all mega-biodiversity areas as well as 'hot-spot' locations with reference to threats to biodiversity, ranging from landscapes to individual species. In addition, for this purpose developing nations rich in agrobiodiversity should levy a 1% cess on all agricultural produce for being credited

to a National Community Gene Fund to be used to recognize and reward the contributions of tribal and rural families to the *in-situ* conservation and enhancement of agro-biodiversity. Such steps will help to restore and revitalize the on-farm genetic conservation and selection-traditions of rural communities.

The role of the international community

To maximize efficiency and return on investment in the Evergreen Revolution, south-south partnership and cooperation in research and development will be essential, especially among nations with related agroecologies. The CGIAR centres should support these emerging regional networks and national systems, pursuing a policy of subcontracting present responsibilities as appropriate. We, the Summit participants, resolve to establish an *International Scientific Steering Committee for Sustainable Food and Nutrition Security*, to provide political leaders with

the scientific framework necessary to achieve food for all. Broader consensus can be fostered through a *Global Coalition for Sustainable Food Security* including farmers' organizations, civil society, academia, corporate sector, service organizations and mass media.

To convert the rhetoric of 'food for all' into reality within a specified time frame, we urge the G-7 and G-15 countries to jointly establish a high level *Steering Committee for Sustainable Food and Nutrition Security*, for which FAO could provide the Secretariat. This unique political body would be fundamental in reaching the shared goals of global food and environmental security, reduced need for emergency aid, enhanced political stability and the development of new markets for trade. This is a responsibility which the political leaders of the G-7 and G-15 countries must accept at the November, 1996, World Food Summit, if we are to enter the new millennium with hopes for a new humane world.

The 11th Himalaya-Karakoram-Tibet Workshop: Conference report

The Himalaya-Karakoram-Tibet (H-K-T) Workshops, held annually since 1985, are a timely response to the growing interest among the international geological community in the Himalaya-Tibetan region (see Sorkhabi¹ for a report on the 10th H-K-T Workshop in Switzerland). These annual meetings are part of an international 'boom', which has made the Himalaya-Tibetan region probably the most prolific field of geologic research among the mountainous terrains of the world. The recent boom in the Himalaya-Tibetan geology is a continual part of more than 150 years of geoscientific research in the Himalaya and south Asia. However, the present diversity and extent of international involvement in the Himalaya-Tibetan geology is unprecedented. The aim of this Himalaya-Tibet saga is no less than unravelling the 'biggest tectonic puzzle of Cenozoic Earth'.

Unlike Britain and continental Europe, whose tradition of geological research in the Himalaya dates back to the first half of the 19th century, North America is relatively a newcomer to the scene. The

first and the only American to have lived and worked in the Himalaya during the 19th century was Alexander Gardiner (1785-1877). Gardiner worked as an army officer in the forces of several kings in Kashmir. His wanderings and missions took him to all of the mountain ranges of the western Himalaya and the Pamir before any European explorer knew of these places. However, Gardiner did not document his observations and travels as a contribution to geographic knowledge².

The 1930s may be regarded as the 'initial pulse' of American studies in the Himalayan region. Helmut de Terra, a German geologist who had immigrated to the US and joined the Carnegie Institution of Washington, carried out pioneering research on the Quaternary geology of the west Himalayan foothills. In the same decade, G. Edwards Lewis and Paul D. Krynine at Yale University began research on the Siwalik formations; the Yale tradition in Siwalik studies (such as the palaeontological work of David Pilbeam) continued through the 1980s. Also in the 1930s, Edwin Colbert exam-

ined the Siwalik vertebrate fossil collection at the American Museum of Natural History in New York.

The 'plate tectonic revolution' of the 1960s motivated tectonic studies of the Himalaya and Tibet as this region came to be regarded as a 'type example' of continent-continent collisional orogenesis. Initially these studies were of 'armchair geophysics' genre, using seismic data and tectonic modelling. Over the past two decades, several institutions in the US have carried out field-based studies in the Himalaya and Tibet, resulting in an increasing number of graduate dissertations. Some of these active groups are those at Dartmouth College (where the late Noye Johnson with his students and colleagues carried out intensive research on the Siwalik Group of Pakistan), Orogen State University, Cornell University, Massachusetts Institute of Technology, University of California and University of Southern California (both at Los Angeles), etc. In addition, there are many individual Himalayan researchers scattered throughout North America. In recent years,

numerous sessions and symposia devoted to the Himalaya and Asia have been held at the annual meetings of American Geophysical Union and Geological Society of America. The US National Science Foundation has also funded numerous research projects on the Himalaya.

The 11th H-K-T Workshop, held at the Du Bois Center of the Northern Arizona University in Flagstaff (Arizona) from April 28 to May 2, 1996, was the first H-K-T Workshop in North America. It was a logical outcome of the increasing involvement of American geoscientists in the Himalaya-Tibetan region. Organized by Allison Macfarlane (George Mason University), Rasoul Sorkhabi (Arizona State University), and Jay Quade (University of Arizona), the Arizona workshop was attended by a total of 115 persons from various countries. Figure 1 shows the geographic distribution of the participants.

Before the workshop began, a one-day field excursion was made to the Grand Canyon on April 28; it was guided by Troy Péwé and Edmund Stump of Arizona State University. After the workshop, another field excursion was made from Flagstaff to Phoenix on May 2. Guided by Stephen Reynolds of Arizona State University, this field excursion was meant to show some geological features of the Basin-and-Range province of Arizona. Each of the field excursions was attended by about 50 people.

The three-day workshop covered various aspects of geological sciences of the Himalayan mountains and the Tibetan Plateau. The morning sessions were devoted to special topics.

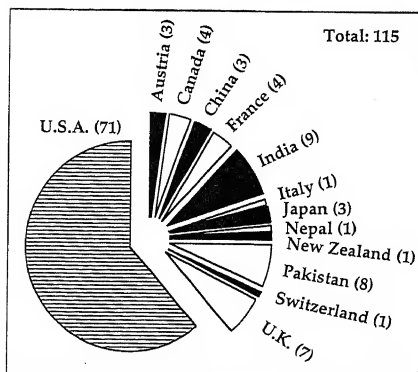


Figure 1. Geographic distribution of the participants at the 11th Himalaya-Karakoram-Tibet Workshop, USA.

The first special session (on 29 April) was on 'Geodynamic models of the Himalaya and Tibet'. From a geological point of view, the Himalaya-Tibetan region is a massive stress system on earth brought about by the head-on collision of the Indian continental plate with Asia during Cenozoic times. While the plate tectonic framework is generally accepted, details of the tectonic evolution and geological processes shaping the Himalaya and Tibet are far from clear. Since India collided with Asia 55 ± 10 million years ago, India has penetrated into Asia for about 3000 metres³. Some of the intriguing questions concern this 'missing continental crust': Was the crust simply shortened by folding and thrusting, and this deformation distributed throughout the Himalaya as well as the entire Tibet? Has the Indochina block (in southeast Asia) extruded southeastward out of the way of an Indian indenter, and thus opened space for the northward motion of India? Has the Indian crust subducted beneath the Tibetan Plateau, thus forming a double-normal thick continental crust? Or has a combination of all these processes taken place? If so, how to quantify their relative dominance in space and through time? Closely linked with the problem of the 'missing continental crust', there is another set of questions concerning the 'uplift of the Roof of the World', which the Himalaya-Tibetan region represents: How and when did the Himalaya and Tibet uplift? What is the nature of lithosphere-aesthenosphere interactions beneath Tibet? Is a hot spot trapped beneath Tibet, thus deriving its thermal uplift as well as crustal extension? In attempts to answer these questions, several tectonic models have been proposed. Speakers in the first special session explored some of these models, and presented new data and ideas. Compared to the 1960s, when plate tectonics was emerging, great progress has been made in obtaining geophysical, geochemical, and geochronological and other quantitative data. Some of the exciting results come from deep seismic crustal profiles of southern Tibet carried out by a US-Chinese collaborative project called INDEPTH (International Deep Profiling of Tibet and the Himalaya)⁴. However, to work out definite, detailed geological scenarios of the Himalaya and Tibet, the ratio of speculation/fact is still high, and we need more data from various sectors

of this vast, less accessible region.

The Himalayan region is sometimes described by geologists as a 'natural laboratory'. They are mountains in the making by vigorous tectonic forces and in the shaping by powerful denudation agents. This provides a common ground for geoscientists in the fields of Quaternary geology, neotectonics and geomorphology to investigate the interactions of tectonic and geomorphic processes in the Himalaya. The second special session (on April 30) focused on the 'Neotectonic and Quaternary geology of the Himalaya and Tibet'. Topics covered in this session included: Applications of global positioning system to quantify the ongoing convergence between India and Asia⁵; determining the recent uplift rates of the Himalaya both from geophysical modelling and direct geodetic measurements; seismic activity (both palaeoseismicity and monitoring of current earthquakes); geomorphic analysis of mountain terrains by space-based imagery techniques; understanding the role of tectonic activities in the geomorphic development of river systems; determining exposure rates of rocks by cosmogenic nuclides; mass wasting and mountain slope processes; mapping and monitoring the dynamics of glaciers; studies of loess; late Cenozoic glaciation record in the Himalaya and Tibet. These topics are not purely scientific; they have important implications for development, construction projects, land-use, hazard mapping, and resource management in the Himalaya and Tibet.

Although the Himalaya-Tibetan region is fundamentally the product of a compressional (crustal shortening) tectonic regime, it has long been known that there are east-west extending grabens in Tibet, suggesting the gravitational collapse of the plateau. Over the past decade, discovery of a basement-cover detachment – the so-called South Tibetan Detachment⁶ – in the Himalaya has drawn a widespread interest among geologists. The South Tibetan Detachment essentially separates the Paleozoic sedimentary sequence of the Tethys Himalaya (on its hanging wall to the north) from the high grade metamorphic and granitic rocks of the Higher Himalaya (on its footwall to the south). More detailed studies have also mapped extensional structures in other geological zones of the Himalaya; for example, S-C fabrics in the Higher Himalayan metamorphic zone, and normal

faults in the foothills of the Himalaya. Interestingly, unlike the east-west spreading grabens of Tibet, the Himalayan extensional structures are parallel to the general east-west strike of the mountains, demonstrating that compression and extension have occurred in the same north-south direction. Available geochronological data also indicate that these two events may be temporally linked to each other. The existence of these two contrasting tectonic regimes in a single orogenic system (which is also, geologically speaking, quite young and still active) provides crucial clues to understanding how tectonic processes deform rocks and develop highlands on the earth as well as put physical caps on their development. Kip Hodges of Massachusetts Institute of Technology remarked that it is no wonder that the highest fault on earth (on top of Mount Everest) is a normal fault. The third special session (on May 1) discussed Himalaya-Tibetan examples of 'Extensional tectonics in a compressional orogenic system'. This area of Himalayan research is expected to attract more attention in the coming years. Especially important will be close examinations of (i) absolute time relations between the activity of the South Tibetan Detachment marking the northern boundary of the Higher Himalayan metamorphic zone and the Main Central Thrust marking its southern boundary; (ii) the relations of Himalayan granites produced by crustal anatexis during the Tertiary both to compressional (hence crustal thickening) tectonics and extensional (hence decompressional melting) tectonics; and (iii) similarities and variations in tectonic style and timing of extensional faults along the strike of the Himalaya.

The afternoon sessions of the workshop covered regional topics. On April 29, two parallel sessions were held on 'Western Himalaya/Karakoram' and 'Sub-Himalaya (Foreland Sediments)'. On April 30, 'The Main Central Thrust Zone' as well as the Poster Session were covered. Finally on May 1, two other parallel sessions were held on 'The Higher Himalayan Crystalline Terrain' and 'Tibet and Trans-Himalaya'. Holding parallel sessions usually has the downside of splitting participants, which may not be welcome by the participants wishing to hear all the lectures. Nevertheless, it was not possible time-wise for the organizers to fit all of the presentations in single sessions

in a three-day workshop. Especially considering the view that in workshops (unlike conferences) more time should be given for questions and discussion. Indeed a novel aspect of the 11th H-K-T Workshop (differing from the usual 15-minute conference presentations) was that each oral presentation was 20 minutes long; furthermore, an open discussion (coordinated by two leaders) for one hour was held at the end of each session. This allowed for more questions, comments, and debates on the topics covered in each session.

A total of 101 presentations were made at the workshop: 71 oral and 30 posters. Geographic distribution of the presentations is shown in Figure 2. It can be seen that 49% of the presentations were on the western Himalaya (Pakistan and India), and that relatively much less research is conducted on the eastern parts of the Himalaya and on the Karakoram-Pamir-Hindu Kush mountains.

It is also informative to consider the distribution of the presentations in terms of the geological divisions of the Himalaya. The Himalaya is divided into five longitudinal zones, separated from each other by major faults⁷. These zones are as follows from north to south: (i) the Trans-Himalaya and the Indus-Tsangpo Suture Zone (the initial plate boundary between India and Asia); (ii) the Tethys (or Tibetan) Himalaya; (iii) the Higher (Greater) Himalaya; (iv) the Lesser (Lower) Himalaya; and (v) the Sub-Himalaya (Outer Himalaya or the Siwalik hills). Figure 3 shows the distribution of the presentations at the workshop according to geological divisions. 33% of the papers were on the Higher Himalayan metamorphic and granitic rocks (i.e. 'hard rocks') towards understanding such processes as metamorphism, crustal anatexis and emplacement of granites, processes of uplift and denudation of deep-seated rocks, timing of major faults and tectonic events in the Higher Himalaya. If the submitted abstracts is taken as a rough measure of research done in the Himalaya (this is a big IF, but the same inference can also be made from considering the subject-matter of Himalayan papers published in general geology journals), it is inferred that relatively less research is conducted in the Tethys Himalaya and the Lesser Himalaya. This is unfortunate because these two sedimentary zones provide valuable information on the Proterozoic, Paleozoic and Mesozoic events.

Only one paper on palaeontology (on the trilobites of the Zaskar region) was presented at the workshop. Much more research in Himalayan stratigraphy and palaeontology is required. The Tethys Himalaya has exposed marine sedimentary formations spanning in stratigraphic time from the trilobites of Cambrian seas until the Great Dying of Cretaceous ammonites. There are also Cenozoic sedimentary formations and fossils in the Himalaya, especially the mammalian fossils in the Siwalik Group, which provide valuable opportunities for examining the evolution of life and environment in the geological past.

Similarly more attention needs to be paid to the Lesser Himalaya. A major problem with this zone has traditionally been the lack of fossils to work out its stratigraphy and relative displacement of rock formations. Perhaps, this is a wrong expectation any more. Applications of high-resolution geochronological and geochemical techniques in conjunction with detailed field mapping in the Lesser Himalaya provide important clues to un-

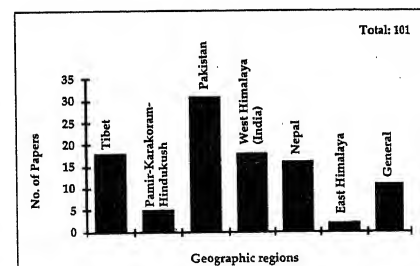


Figure 2. Presentations (both oral and poster) at the 11th Himalaya-Karakoram-Tibet Workshop according to geographic regions of research (Tibet is here considered as a geopolitical region).

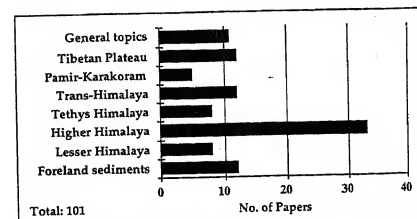


Figure 3. Presentations (both oral and poster) at the 11th Himalaya-Karakoram-Tibet Workshop according to the geological divisions (Tibetan Plateau is here considered as a geological zone to the north of Trans-Himalaya).

derstanding the Precambrian history of the earth. Moreover, the Lesser Himalaya is the most populous zone of the Himalaya, containing forests, agricultural lands, human settlements, infrastructures, etc. Research in such fields as geomorphology and neotectonics of the Lesser Himalaya would be important in terms of understanding the mountain-human interactions.

Selected, refereed papers resulting from the workshop will be edited by the organizers and published as a volume of the *Geological Society of America Special Paper*, and also possibly in a special volume of the journal *Tectonophysics*. Financial support for the Workshop came partly from registration fees and partly from a grant from the Continental Dynamics Program of the US National Science Foundation (NSF). The NSF grant mainly supported travel funds (some partial support and some full funds) for 24 researchers from the Himalayan countries (China, India, Nepal, and Pakistan) and students from Europe. The Indian participants at the workshop were from the

Wadia Institute of Himalayan Geology (Dehra Dun) and University of Roorkee. Overall, 28 of the participants were originally from the Himalayan countries (including those residing in the US or Europe). Indeed, one of the successes of the workshop was that it could provide financial support for native researchers in the Himalaya to attend this international meeting. (Nearly 100 applications were received for travel award; it was not financially possible for the committee on travel awards to respond positively to all of these requests.) Participation of researchers from the Himalayan countries is important for the H-K-T Workshops; however, not all organizers have been or will be able to obtain the necessary funds (especially in view of shrinking government budget for science in recent years). One solution is to hold the H-K-T Workshops frequently in the Himalayan countries (so far, only one of the workshops has been held in the Himalayan region, namely the Kathmandu Workshop in 1994). This solution was suggested in Flagstaff during the planning session for

the 1997 H-K-T Workshop, and deserves more attention.

The 12th H-K-T Workshop will be held in Rome (Italy) in April 1997 on the occasion of the 100th anniversary of birth of Ardito Desio, a geologist who carried out pioneering research in the Karakoram mountains.

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Chaos – A new paradigm for comprehending nature

The earliest study of what is today known as *deterministic chaos* dates back to the paper entitled 'Deterministic Nonperiodic Flow' by E. N. Lorenz¹ in 1963. However the concepts of chaos took roots only in the seventies. By the eighties there was an exponential growth in the numbers of research articles and books. Journals devoted entirely to this dynamic new concept were started, and the number keeps increasing – especially if one considers the related areas of fractals and complexity.

A four-day Workshop on Complex Systems (chaos) was organized under the auspices of CSIR Technical Advisory Board (Physical, Environmental & Earth Sciences), hosted by C-MMACS, at C-MMACS/NAL from June 26 to 29, 1996. The motivation for holding this Workshop stemmed from the view that it would be desirable to empower CSIR scientists with a working knowledge and hands-on experience in this fascinating, new, dynamic area of physics so that they may make creative applications of

these paradigms to new problems in their respective fields. Interested scientists and students from outside CSIR were also included among the participants to ensure benefits on a wider scale. The participants had a background in diverse areas of physics, biology, engineering, mathematics, computer science, etc.

Deterministic chaos is a nonlinear phenomenon and is easily understood by studying the behaviour of the simple one-dimensional nonlinear map², the so-called logistic map, $x_{n+1} = \mu x_n(1 - x_n)$. This is so simple that one can programme it on a hand-held calculator without much trouble. A physical understanding of this equation can be gained by considering the variable x_n to represent the normalized population of some organism in the n th generation, x_{n+1} becoming the population in the next generation. The model is discrete in time and assumes that the size of the present population depends only on the size in the previous generation (some insect species do approximate such simple dynamics). The first factor on the

right hand side, μx_n , is the growth term and would lead to a runaway exponential increase but for the inclusion of the second factor. The latter, μx_n^2 , represents the decay term and the limit to growth such as, for example, controlled by a finite amount of food supply. Here, we treat x_n as a normalized variable (a proportion of some maximal population) and consider values $0 < x_n < 1$. The model exhibits a range of dynamical behaviours for different values of the parameter, μ . Figure 1 shows a sequence of values of x_n , with $n = 1, 2, 3, \dots$, namely an orbit of the system, for $\mu = 4$, and a similar behaviour is obtained regardless of any specific initial condition x_0 . There is no regularity in the sequence: it looks quite random in character and is, in fact, quite unpredictable. The unpredictability is evident when one starts off with two initial conditions which are very slightly different from each other. After just a few iterations, the two sequences bear no resemblance whatsoever, and can differ from each other by arbitrary amounts within

the general confinement. All the sequences are constrained to lie between 0 and 1, so the difference in absolute terms may not be large, but locally, nearby orbits diverge from each other at *exponential* rates. We have a situation where although the equation is deterministic, the outcome is neither regular nor predictable in the long run. This is characteristic of deterministic chaos.

In the early part of this century, a major paradigm shift occurred with the discovery of quantum theory, which introduced us to the uncertainty principle: that both of a pair of complementary dynamical variables (observables), such as position and momentum, cannot be measured with arbitrary accuracy simultaneously. This led to the formulation of the mathematical apparatus of the theory in terms of probabilities. However, the probability functions themselves were evolved using deterministic equations in the theory. The philosophical ramification of all this has been, and continues to be, debated extensively.

In the past decade, *chaos theory* has again confronted us with the question of limits to predictability³: it says that, as long as the equations of evolution are nonlinear, even if deterministic, any imprecision in initial measurements, can, during subsequent evolution, get magnified exponentially fast, so that no predictions can be made with sufficient accuracy in the long run. This is a statement with far reaching implications. Almost all systems are nonlinear in nature: linearity is most often a convenient approximation. However, until recently, most developments in science and technology have been based on this approximation. This is understandable because linear theory is analytically tractable whereas nonlinearity, in general, is not. It is only with the advent of computers that it has become possible to tackle nonlinearity directly, using numerical tools.

Quite apart from the philosophical implications, beautiful results and techniques have been spawned by the chaos theory⁴. The hallmark of chaos, namely the sensitivity to initial conditions or the exponential divergence of nearby trajectories can be quantified by the rate of divergence, which is termed the Lyapunov exponent. A positive exponent implies exponential separation of trajectories or chaos. There are as many exponents as

there are phase space dimensions and, while divergence occurs in some directions, contraction can occur along other directions. For conservative systems, the net expansion is zero whereas in dissipative systems volumes contract (we deal only with the latter case here). Thus, even though nearby trajectories diverge, globally all trajectories remain confined to some subset of the phase space. This is achieved by a process of folding back of the trajectories at the boundaries. This process of local divergence and global confinement through 'stretching and folding' is typical of systems where the motion is chaotic (a good visual mnemonic of such a process is the kneading of dough by a baker). Coupled with the contraction of phase space volumes, what results in phase space is an *attractor*, namely the orbit to which progression from any initial conditions eventually converge. When the motion is chaotic, the attractor often has a fractal geometry, and is termed *strange*. Systems can have different strange attractors and fractal (non-integer) dimensions have to be used to characterize them. Nonlinear dissipative systems can have simple attractors as well, such as fixed points (steady states) or limit cycles.

A remarkable result from chaos theory has been the discovery of universal routes to chaos. As a control parameter (as for

example μ in the map above) is varied, one observes qualitative changes in the dynamical behaviour and the phase space dynamics switches between the different attractors in a universal manner. Three well-defined universal routes to chaos have been documented in various experiments: the Feigenbaum scenario or the period-doubling route to chaos, intermittency and the Ruelle-Takens or strange attractor scenario. The period doubling route is characterized by a set of universal numbers, which relate to the way in which aperiodic behaviour is approached. As the parameter is varied, one observes period doubling, i.e. from a steady state to a periodic motion with period (say) T , to $2T$, to $4T$, $8T$, ..., $2^n T$ and thus on to an aperiodic or chaotic behaviour. The parameter windows in which successive periods exist keep getting smaller and the ratio of two such successive window lengths approaches a universal number, the so-called Feigenbaum constant, which has the numerical value 4.669.... In essence, what happens is that, as the parameter varies, one limit cycle becomes unstable and a new limit cycle of twice the period is stabilized. Ultimately no cycle is stable but all exist, albeit as unstable ones. In other words, chaos can be considered as a closure of unstable periodic orbits. It is possible then to visualize chaotic dynamics as one in

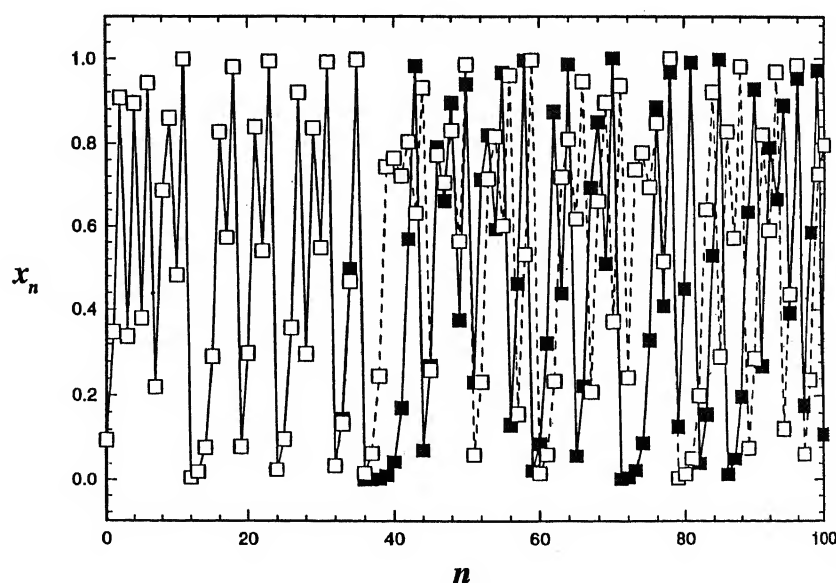


Figure 1. Sensitivity to initial conditions, hallmark of *deterministic chaos*: two sequences of iterates of logistic map differing only in the twelfth decimal place in the initial conditions.

which the phase space orbit gets kicked from one unstable orbit to another and keeps wandering in the skeleton formed by the unstable periodic orbits, unable to settle down on any of them.

Dynamical chaos can be exploited. The knowledge that there are unstable periodic orbits within chaotic regions has been used to devise beautiful control algorithms, where a nonlinear dynamical system can be made to have a desired dynamical behaviour corresponding to a particular periodic orbit. Since the system is extremely sensitive, small parameter changes are enough to manipulate the dynamics and it is possible to design a series of such small parametric perturbations to keep the system around a periodic orbit of interest. This possibility vests chaos with a desirable feature where it provides for versatile design options. For, one can thus control, simply by tuning parameters involving very small changes, and switch the system from one periodic behaviour to another, and no major design modifications are called for!

Experimental data from biological systems appear to suggest that nature has indeed incorporated some of these aspects of chaos into its engineering designs. EEG data from individuals in different stages of consciousness indicate that wakefulness is characterized by chaotic dynamics whereas different stages of sleep are marked by different periodic oscillations. Furthermore, pathological conditions in brain, for example epileptic states, are marked by periodic behaviour.

One of the most important features of deterministic chaos is that it can arise in systems of low dimensionality. In discrete systems, one-dimensional maps (if non-invertible; else two-dimensional) exhibit chaos and, in continuous systems, three variables, coupled ordinary differential equations are sufficient (nonlinearity being, of course, a prerequisite). Quite obviously, the Newtonian world view – Laplacian determinism – which attributed randomness to high dimensional, complex systems that are 'difficult' to be modelled in complete detail seems unnecessary in hindsight and recent attempts to model complex systems such as the weather or climate, biological systems like the heart or brain, etc. using low-dimensional deterministic nonlinear equations, have attained some measure of success. Simultaneously, data from such systems when analysed to ascertain

the actual effective dimensionality of those systems reveal them to be indeed low-dimensional in very many cases.

The study of complex phenomena is not restricted to the dynamical regime. A significant development in the study of complex systems was the introduction of the concept of self-organized criticality by Bak, Tang and Wiesenfeld⁵ (BTW) in 1987. Critical points are familiar from the study of phase transitions and they are approached by the tuning of an external parameter such as, for example, temperature in the case of water-to-ice transition. However, in self-organized criticality, there is no need to tune any parameter and the system is constantly maintained in the critical state by processes of self-organization: these are far-from-equilibrium systems⁶. The paradigmatic model that BTW studied is that of sandpiles: sand is added a grain at a time, and the slope of the pile keeps fluctuating between a minimum and a maximum value within a small range. In the steady state, the system is marginally stable and avalanches of all sizes occur as the slope keeps fluctuating. The model which is a simplified description of systems with extended spatial degrees of freedom, has generated considerable interest. An important natural phenomenon that is seen to correspond to this class of systems is that of earthquakes with their associated stick-slip dynamics.

Sandpiles are simple, paradigmatic models for complex phenomena, but, at the same time, they themselves are very complex systems from another perspective. In fact, they have been referred to as a 'new' state of matter, the *granular state*, which is intermediate between solids and liquids, having properties such as dilatancy, hysteresis, etc. A unique characteristic of sand is to sustain the evolution of bridges and arches which, unlike in liquids and gases, do not get thermally averaged away and play a significant role in stasis and dynamics. For example, due to these local formations, the stress distribution at the bottom of a sandpile is quite complicated. Again, the packing configurations are significantly affected by the presence of such formations.

The problem of turbulence is one that continues to challenge physicists. In the light of new developments, some of which have been described above, this is now described as 'fully developed chaos'. New techniques of analysis from chaos theory

have been applied to turbulence data to gain some new perspectives.

Among the new techniques that have come up, a very useful one is that of phase space reconstruction. Quite often, in experimental situations, only one, or at most a few, of the variables involved in the dynamics can be measured. In the absence of a knowledge of the complete set of phase space variables, it is still possible to construct a pseudo-phase space which is topologically equivalent to the (unknown) original. The set of new coordinates for such a phase space is constructed from the available one by using time-shifted, lagged values: if $v(1), v(2), \dots$ are the measurements of a variable v at time intervals $1, 2, \dots$, the time series for the second coordinate can be taken as $v(1 + \tau), v(2 + \tau), \dots$, with τ , an integer multiple of sampling time, chosen in an appropriate manner so as to avoid redundancy. The phase space thus reconstructed can be used to estimate dynamically relevant quantities like Lyapunov exponents, fractal dimensions, unstable periodic orbits, etc.

The widespread availability of powerful electronic computers has played a major role in many of these developments by making the simulation of complex systems tractable. Many studies of complex behaviour can be carried out with simple models that capture the essential features. Cellular automata – where space, time and all variables are taken to have only integer values – have been extensively used to model a variety of such systems. Coupled-map lattice models – where space and time are discrete – also provide a numerical methodology capable of displaying rich phenomena.

It is this fascinating, frontier area of physics that was the subject matter of the workshop. The workshop began with a keynote lecture by R. Ramaswamy (JNU, Delhi). Dissipative systems were reviewed in two lectures by V. Balakrishnan, (IIT-Madras). Neelima Gupte (IIT-Madras) gave three talks. In the first one, she introduced the basic concepts of fractals, the fascinating field of new geometry, which appears to describe natural objects such as trees, clouds, coastlines, mountain profiles, etc., all of which have non-integer dimensions. Her second lecture dealt with the control of chaos and the third was on synchronization. The latter has important applications, particularly with respect to encryption of

messages (of importance in defence) where the messages are masked by synchronizing with chaotic waveforms.

T. R. Krishna Mohan (C-MMACS, Bangalore) explained the procedures for reconstruction of a phase space from single variable time series and the estimation of correlation dimension (Grassberger-Procaccia algorithm⁷) and Lyapunov exponents from the phase space dynamics. Pradhan (NIMHANS, Bangalore) discussed and explained the problems associated with ascertaining whether a time series originates from nonlinear, deterministic evolution or from a linear, stochastic process which can give rise to coloured noise having similar spectral properties. Prabhakar Vaidya (Washington State University, now visiting C-MMACS) gave a talk on methods that can be used for unambiguously confirming the presence of a nonlinear, deterministic process in time series; the methods he spoke on (trans-spectral coherence) are related to higher order spectral methods and can also be used in connection with premonition of chaos, i.e. to identify possible transition to chaotic states.

Deepak Dhar (TIFR, Mumbai) gave two lectures on self-organized criticality and sandpile models; he showed the relationship of abelian sandpile models to other related models such as Potts model, Voter model, Takayasu aggregation model and river networks.

Anita Mehta (S. N. Bose Institute for Basic Sciences, Calcutta) spoke on the

complexity of granular materials, namely sand, and the relevance of ideas such as self-organized criticality to the physics of actual sandpiles. Sudeshna Sinha (Indian Institute of Astrophysics, Bangalore) discussed the general strategies of modelling spatio-temporal phenomena by coupled-map lattice models.

Three afternoon sessions were devoted to experiments. A variety of PC-based computer programs were made available to participants in order to help them understand the various concepts covered during the lectures, and to enable them further explore different aspects of chaotic dynamics and fractal geometry through numerical experiments. There were also experiments using electronic circuits to show the period doubling route to chaos, as well as strange attractors. A practical realization of the phenomenon of chaotic synchronization using two circuits was shown, and the ideas of secure communication using chaotic masking was demonstrated by Manu and Kapilanjani Krishan of Khalsa College, New Delhi.

Three evening colloquium talks marked the conclusion of the workshop activities on each day. Anita Mehta gave a general overview of the physics of sand, of the physics of granular state, in her colloquium talk, 'Probing Sand'. Rahul Pandit (IISc, Bangalore) talked on homogeneous, isotropic turbulence with emphasis on the scaling of velocity structure functions, on the multiscaling and self-similarity aspects. In another, R. Narasimha (IISc and

JNCASR, Bangalore) talked on aspects of clouds as complex systems with focus on understanding of entrainment process in tall cumulus clouds where horizontal diffusion is practically nil and vertical diffusion is practically infinite! He described experiments being carried out at IISc (with Prabhu and Bhat) to study the mechanism of this strange behaviour. The large-scale vortical structures in the flow 'engulf' ambient fluid from the surroundings in the first process of entrainment, followed by 'mingling' of the engulfed fluid into the core flow and, finally, it is 'mixed' in at the molecular level. The experiments are designed to study the modifications brought about by local heating which takes place, for example, by the release of latent heat during condensation, in clouds.

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OPINION

Monsoon: A bioclimatologist's point of view

V. M. Meher-Homji

A plant geographer's main concern with the climate is to seek correlations with the vegetation. Precipitation, temperature and evapotranspiration have been commonly used. Lesser known factors like the régime (season of occurrence of rains) have been developed here for a better understanding of eco-diversity. Briefly discussed are the different origins of rainfall in India, the variability in the climatic factors and the transition from the Mediterranean régime to the west of the Indian sub-continent to the tropical monsoon type in the peninsula, across the Thar desert. A critical review is presented on palaeopalynological studies that tried to make out monsoon fluctuations during the Holocene. Deforestation may affect the local rainfall pattern without there being a change in the intensity of monsoon as a planetary phenomenon.

The monsoon, the very pulse of the Indian economy, is a word of Arabic origin

'mausim' meaning season. The term implies the seasonal reversal of the wind

systems on which depended the marine navigation for the purpose of trade. Since

the south-west winds are accompanied by rains, the layman tends to equate the monsoon with rainfall.

Different meteorological origins of rainfall

In a country like India, rainfall could be of different origin in meteorological sense. The south-west monsoon, the major source of moisture in the Indian peninsula, prevails from May–June to September–October. This system very much depends on the sea surface temperature and pressure. El Nino years witness reversal of normal conditions and consequently the monsoon fails in India.

There are two branches of the SW monsoon, the Bay of Bengal branch arriving earlier than the Arabian sea branch. Calcutta receives rain in the last week of May but Bombay only towards the second week of June. After September with the shift in the heat belt from Rajasthan–Punjab to the equator, the winds change direction; from mid-October to December dominate the north-east winds. The rains received in the Coromandel–Circar coastal region during this season are assigned to the NE monsoon. Earlier, the geographers believed that the winds from the north blowing over the Bay of Bengal picked up moisture and provided rains to coastal Tamil Nadu and Andhra Pradesh during the cooler season. Hence the term NE monsoon or retreating monsoon. However, when the winds are blowing from the north-east, the weather remains bright and clear. These are the depressions and cyclones formed during this season in the Bay which bring rains on the east coast. As the frequency of depression formation is highly variable, the rainfall also fluctuates a good deal, from 600 mm to 2000 mm at Madras.

Rainfall may be of orographic origin in the hills. The principle is that an air-mass striking a vertical obstacle like a mountain tends to rise. Finally, the rainfall may be of convectional origin. Due to excessive heating of certain pockets of land, especially in the pre-monsoon months, rising currents of air are set into motion taking the cloud moisture to considerable heights. Perhaps, it is the convectional type of rainfall that is most affected by the forest cover. Historical records reveal that the Chota Nagpur plateau of Bihar used to receive regular afternoon showers in April–May which

favoured tea gardens but as probable result of massive deforestation carried out towards the turn of the century, this instability rain disappeared and it is no longer possible to grow tea in the area. Yet another example is that of Udhagamandalam (Ooty) which had 375 rainy days for the 5-year period 1870–1874, excluding the SW monsoon months June to August. For the 5-year period a century later, applying the same criteria, the number of rainy days has declined to 270 (ref. 1). It would be an interesting exercise to work out the contribution of these different categories of rainfall to the annual total.

Régimes of rainfall

Though India is a tropical country, the season(s) of occurrence of rains vary from one part to the other. Mangalore (Figure 1) represents the typical tropical *régime* with rains beginning in late May and terminating after October, the peak being in July. Thus the rainfall curve maintains a perfect symmetry in the course of the year. Coming to the east coast of Tamil Nadu, at Tirunelveli (Figure 2), the symmetry of the curve is lost as the bulk of rains are received in October–November from the so-called NE monsoon. The rainfall is not only low but also erratic during the SW monsoon season over the Coromandel coast. In fact the character of rainfall in June, July, August at Madras and Pondicherry suggests that these are convectional showers (thunderstorms); over 85 per cent of rains occur between sunset and sunrise in short spells mostly accompanied by thunder and lightening.

Mysore (Figure 3) also experiences little rains during the SW monsoon; the *régime* is characterized by two peaks, one in April (due to convection) and the other in October (depressions and convection). The two peaks are reminiscent of the equatorial *régime* of the stations close to the equator like Colombo. Udhagamandalam (Figure 4) has a three-peak *régime*: July (SW monsoon), besides April and October. These few samples illustrate the range of *régime* in India^{2,3}.

Régimes and vegetation

Realizing that the vegetation is determined by several variables of climatic factors, Köppen⁴ used the data of precipitation

together with the temperature to establish the boundaries between the arid, semi-arid and humid climates and the corresponding vegetation. Given that the precipitation

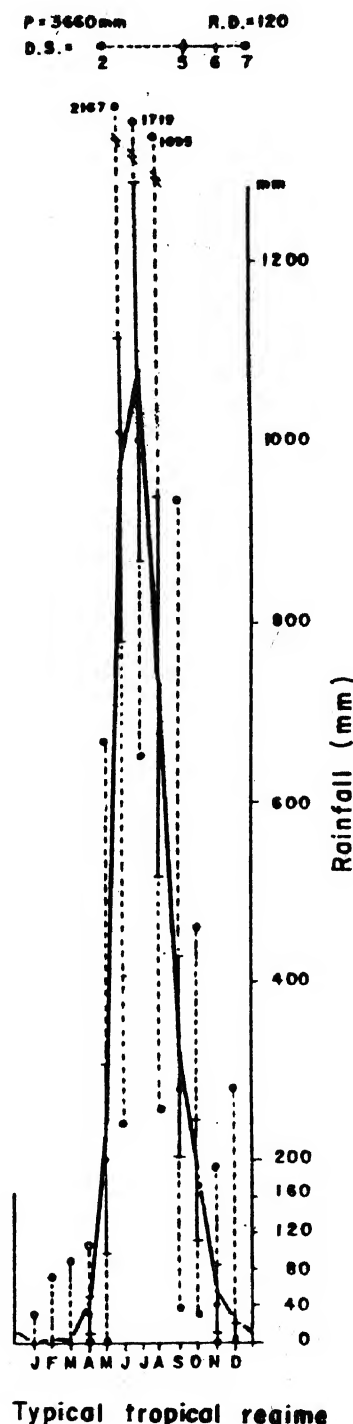


Figure 1. Rainfall *régime* at Mangalore (alt = 22 m). Δ = Rainfall curve; \circ = Octile values; P = Rainfall; RD = Number of rainy days; DS = Length of dry period in months.

is more effective if it occurs during winter than in summer, he assigned different weightage to the areas of summer rainfall and winter rainfall.

We wish to highlight the role of *régime* factor, i.e. of the season of occurrence of rains with reference to some noteworthy examples of vegetation in India. The forest map (Figure 5) shows the dominance of sal (*Shorea robusta*) in the eastern half of the peninsula and that of teak (*Tectona grandis*) in the western half. In terms of rainfall quantum, temperature, length of the dry period there is not much difference in the ranges of these factors. What is important is the timely arrival of rains when the viable seeds of sal are available. The seeds have a short

period of viability of about ten days. If the rains are not in time, the germination fails. The rains which set in earlier in the eastern part of the peninsula favour the sal germination. As rains are delayed in the western counterpart, sal gradually disappears westwards and the dominance is taken by teak. In coastal plains of Tamil Nadu prevails a very unique vegetation type termed as the *tropical dry evergreen forest*. Occurring in the physiognomic form of scrub jungle, it is deprived of the typical species of the deciduous forest like teak, sal, rosewood and many more. The distribution of this type coincides with the zone experiencing the dissymmetric type of rainfall *régime* illustrated in Figure 2.

The economically valuable red sanders tree (*Pterocarpus santalinus*) is confined to the Cuddapah basin and the Nallamalai Hills in Andhra Pradesh. Its endemic occurrence corresponds to the zone where the transition is taking place from the dissymmetric *régime* of Nellore on the Coromandel coast to the typical tropical *régime* of Kurnool on the Deccan plateau (Figure 6) (ref. 5). These few instances bring out the significance of rainfall

régime factor in bioclimatology, a factor ignored both in geography and forestry.

Variability of monsoon

The inter-yearly variability in the wake of the vagaries of monsoon distorts the image of the climate based on averages. The variations in the annual quantum of rainfall are too familiar to merit further discussion. The consequent fluctuations in the length of the dry period though significant in plant geography have been almost overlooked. At Kodaikanal, the majority of individual years experience a dry period of 1 to 4 months, yet on the basis of averages, the dry season does not come out as the dry spells occur in different months in different years. New Delhi, with an average dry period lasting for 9 months, has the range of dryness of 7 to 11 months.

In other cases, the variations involve

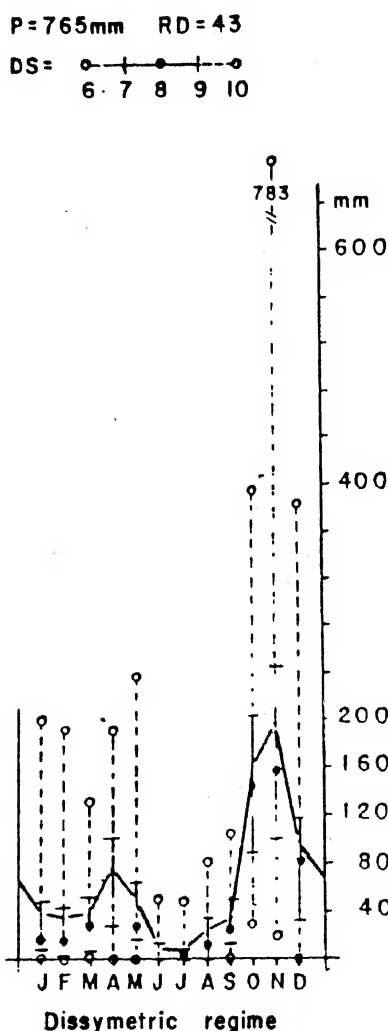


Figure 2. Rainfall *régime* at Tirunelveli (alt = 42 m). Δ = Rainfall curve; \circ = Octile values; P = Rainfall; RD = Number of rainy days; DS = Length of dry period in months.

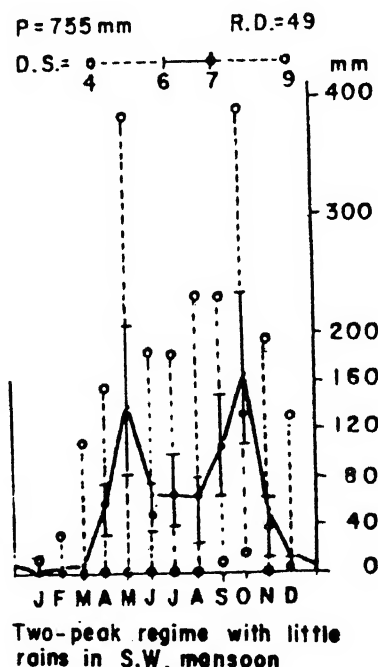


Figure 3. Rainfall *régime* at Mysore (alt = 767 m). Δ = Rainfall curve; \circ = Octile values; P = Rainfall; RD = Number of rainy days; DS = Length of dry period in months.

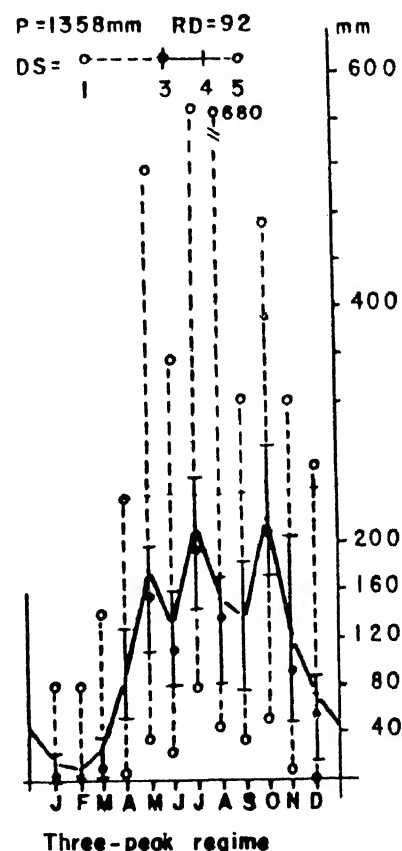


Figure 4. Rainfall *régime* at Udhagamandalam (alt = 2249 m). Δ = Rainfall curve; \circ = Octile values; P = Rainfall; RD = Number of rainy days; DS = Length of dry period in months.

the *régime*. Some stations in the northern and north-western parts of the sub-continent present the average *régime* of Mediterranean type with winter-spring rains brought on by the western disturbances, and summer dryness. However, the *régime* tends to vary from year to year as exemplified by the climate dia-

grams of Peshawar (Figure 7). The interplay between the two rain-bearing meteorological phenomena, — western disturbances and summer monsoon, in the Indo-Pakistan arid zone results in a series of *régimes* shown in Figure 7. It is in this desertic tract of the Thar and adjoining regions that the transition takes place from

the Mediterranean *régime* of countries like Iran and Afghanistan to the tropical monsoon *régime* of peninsular India.

Palynology and the intensity of monsoon during the Holocene

Palynological data have been used to

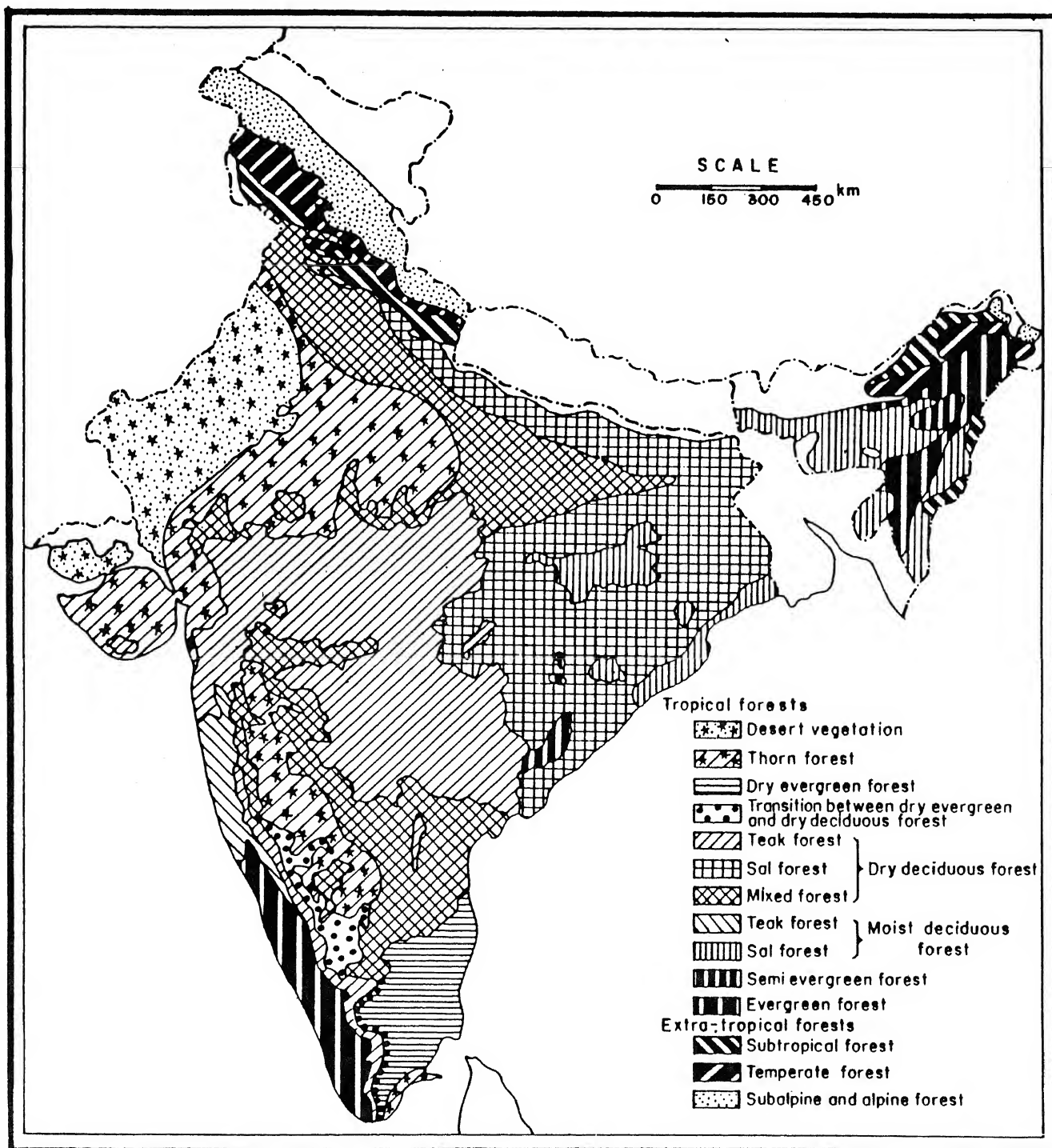


Figure 5. Forest types of India.

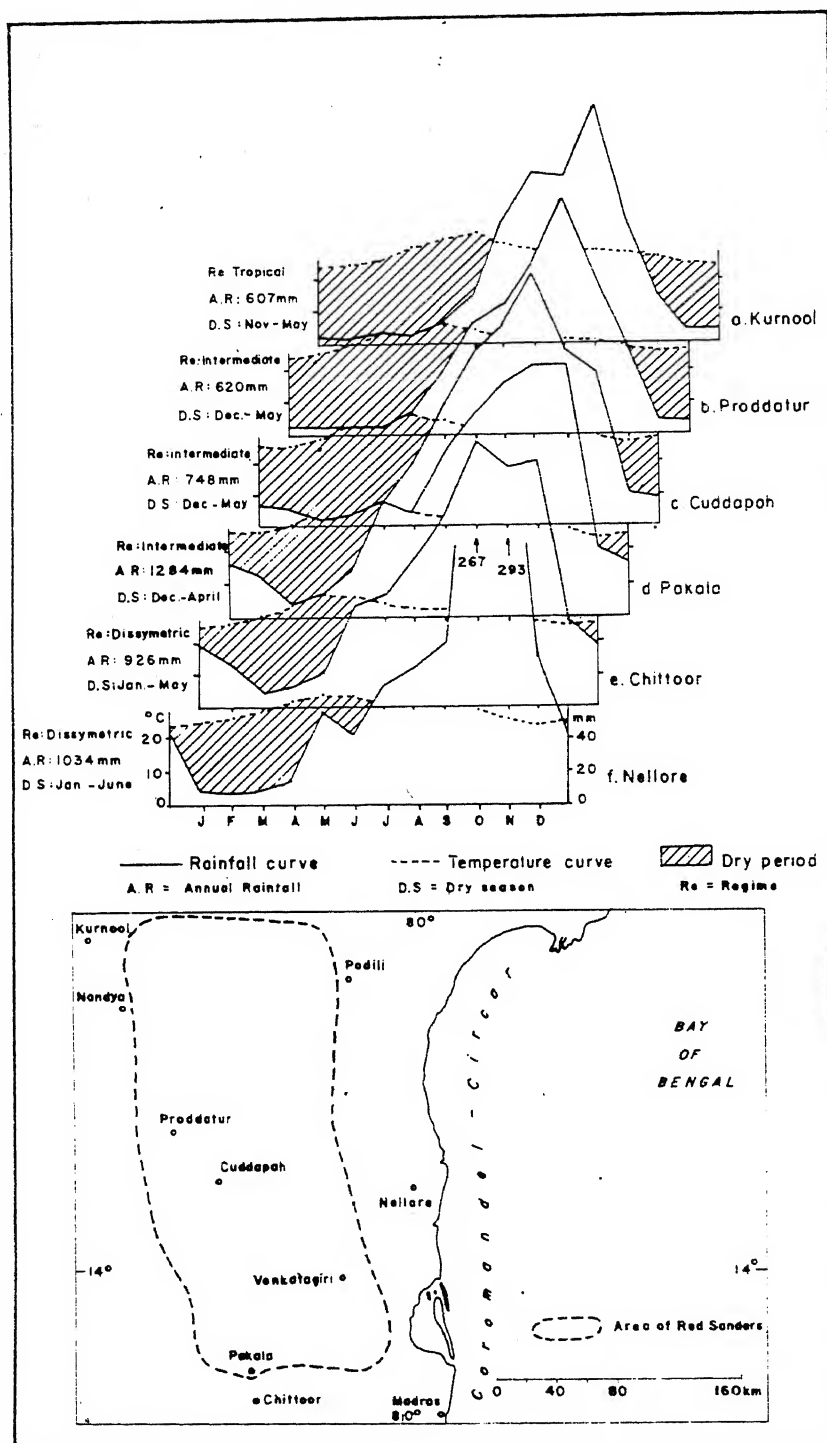


Figure 6. Climate diagrams and area of the red sanders.

decipher the strength of monsoon, weak or strong, within the last 10,000 years. Gurdip Singh *et al.*⁶, based on the analysis of pollen grains of sediments of lakes in

Rajasthan suggested alternation of several relatively humid phases with drier ones during the Holocene. However, the species selected as markers of stronger mon-

soon activity are unfortunately not very fidel indicators of humid conditions⁷⁻⁹. If the rainfall had taken a distinct upward trend, why do the pollen of deciduous forest species of the Aravallis find no representation in the profile of the Sambhar lake?

Caratini *et al.*¹⁰ analysing two marine cores collected from the inner shelf off Karwar opposite the mouth of the Kalinadi for their pollen content observed a change in the vegetation pattern beginning around 3200 years BP, getting more pronounced a millennium later and stabilizing thereafter. A marked decline in the pollen of the evergreen, deciduous and mangrove forest species in the favour of grassland species was taken as an indication of onset of a less humid phase linked to a weaker monsoon. However, the anthropogenic interference cannot be ruled out altogether in bringing about the vegetation change. Man's activity started with iron implements around 1000 BC or even earlier with slash and burn agriculture^{11,12}. Grasslands are essentially the result of passage of fire at low and middle elevation. Paddy cultivation in the estuaries was probably the reason for the decrease of mangroves¹¹.

The decline in rainfall need not necessarily imply a weakening of monsoon activity; large scale deforestation may affect the microclimate and local rainfall. The link between forests and rainfall has been a controversial issue and a debated topic. Gadgil and Prasad¹³ have stressed the use of realistic models capable of simulating the various effects of deforestation such as changes in albedo and soil moisture. Their computer experiments have shown that model simulated climates are influenced by land-surface boundary conditions. The areas most likely to undergo desertification in view of deforestation and the attendant biogeographic feedbacks are those in the vicinity of arid zones. The increasing marine influence noted by Caratini *et al.*¹⁰ through proliferation of marine organisms like copepods, dinoflagellates, foraminifera and organic carbon of marine origin around 2200 years BP was likely due to a decrease in the flow of fresh water, in turn probably linked to massive deforestation.

In conclusion, the archaeological evidence of increasing human influence on the vegetation cover and the role of forests on the local climate deserve consideration

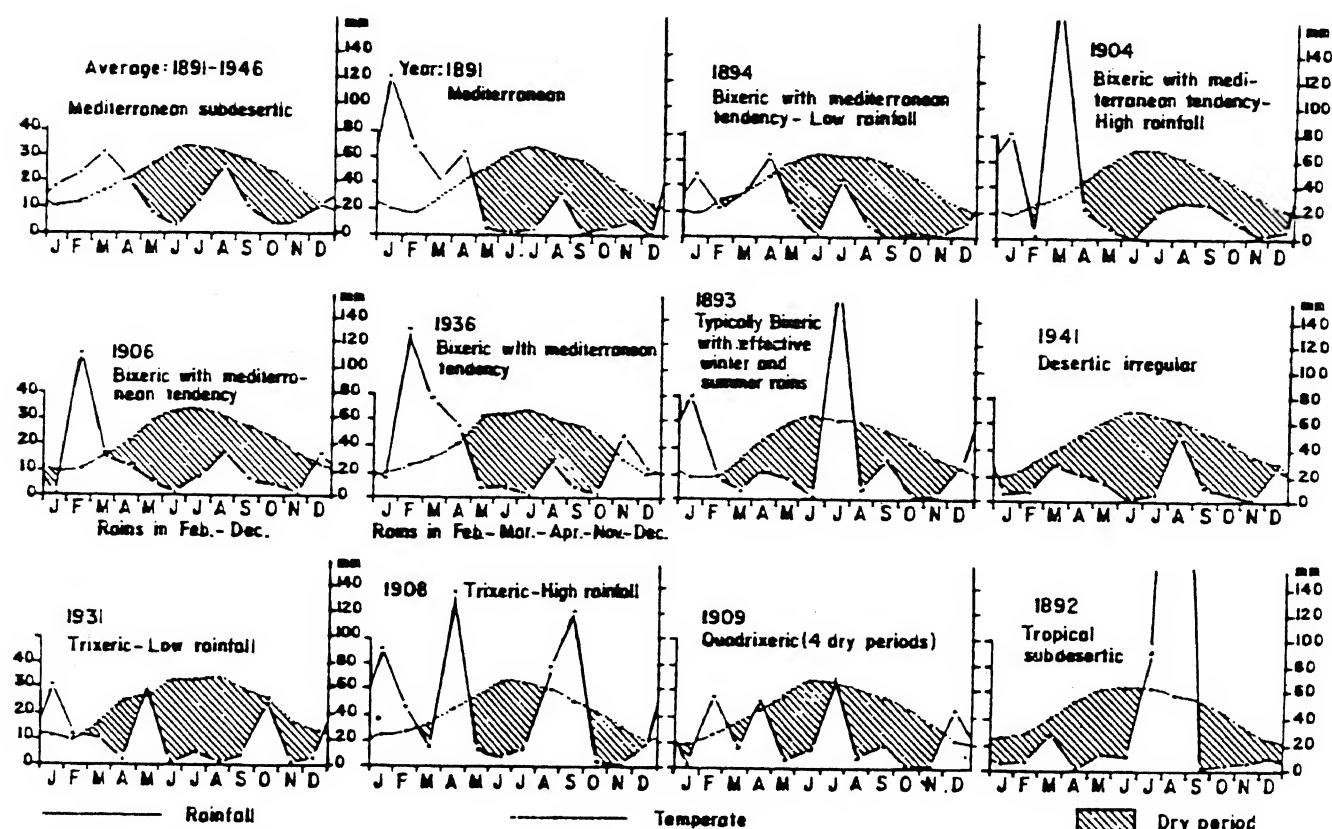


Figure 7. Inter-yearly variations in régime of Peshawar.

while discussing climatic fluctuations and monsoon behaviour in the later half of the Holocene.

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Peroxidase activity in relation to *in vitro* rhizogenesis and precocious flowering in *Bambusa arundinacea*

Bambusa arundinacea, a multipurpose, monocarpic, woody perennial bamboo, exhibits gregarious flowering once after a prolonged intermast period of 30–45 years¹. Incidentally, we noticed *in vitro* flowering in one out of ten seedling lines of *B. arundinacea*. The physiological basis of such flowering in bamboos has not been investigated. However, Gaspar, while studying variation in peroxidase activity in relation to *in vitro* rhizogenesis in several plant species, proposed that rhizogenesis and flowering are two antagonistic phenomena, preceding respective minimum and maximum levels of peroxidase activity^{2,3}. Therefore, we sequentially followed peroxidase activity *vis-à-vis* the *in vitro* rhizogenesis and precocious flowering in *B. arundinacea*.

Ten subculture-cycle-old clonal explants from the seedling line of *B. arundinacea*, which exhibited *in vitro* precocious flowering during the 6–7 subculture cycle, were chosen for the present study. The clonal explants were maintained on 0.7% agarified and 3% sucrose enriched MS medium⁴ supplemented with 2 mg l⁻¹ BA for shoot multiplication and 3 mg l⁻¹ NAA for rooting of multiplied

shoots. pH of the medium was adjusted to 5.8 before autoclaving, and the cultures were kept at 25 ± 2°C under 16 h photoperiod from white fluorescent tubes. Root and flower primordia were visible within 10 days and after 45 days of inoculation on rooting medium respectively. The sampling for peroxidase activity⁵ and protein content⁶ was done daily for an initial ten days and on every third day after eighteenth day onwards until the appearance of flower primordia, taking three samples each of 100 mg shoots from cultures maintained on rooting medium. The data presented here represent mean of three experiments, using independent sets of proliferated shoots, and have been subjected to statistical analysis, employing Student's *t* test at *p* = 0.05 level of significance for comparing mean values of peroxidase activity recorded at various days after inoculation.

The peroxidase activity of *in vitro* proliferated shoots transferred on rooting medium exhibited a definite pattern, attaining significant minimum values at 7 days and 42 days; but the *in vitro* root and flower primordia were visible at 10 days and 45 days respectively after inoculation

(Figure 1). In fact, *de novo* organogenesis is a complex phenomenon, involving a cascade of cytological events, e.g. cell division, elongation and maturation (differentiation). The observed depression in peroxidase activity prior to appearance of root or flower primordia presumably indicates initial events such as cell division and elongation, whereas its subsequent elevation until formation of roots and flowers (Figures 1 and 2) seems to be associated with lignification of differentiating cells, i.e. cell maturation. The role of peroxidase in lignification of cells is well established as it catalyses the oxidation of cinnamyl alcohols into lignin precursors⁷. There is no report in the

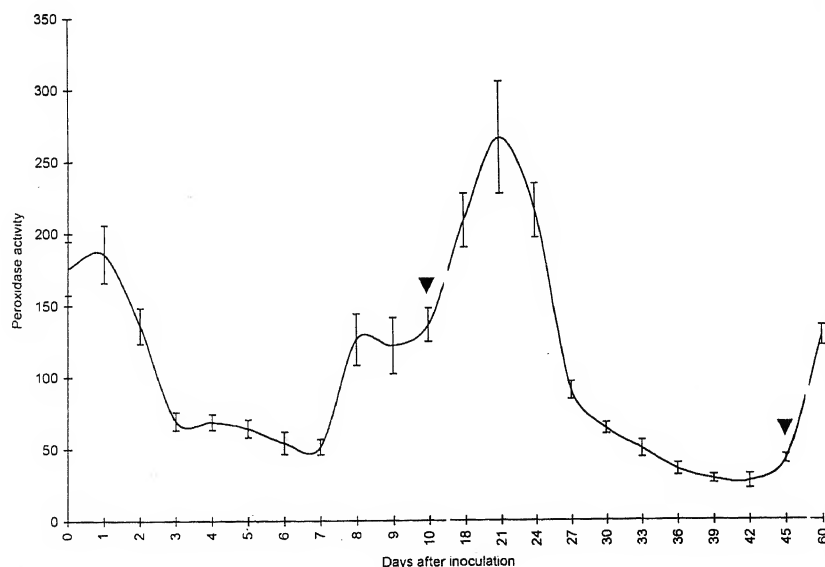


Figure 1. Peroxidase activity [A470 min⁻¹ (mg protein)⁻¹] in relation to *in vitro* rhizogenesis and precocious flowering in *Bambusa arundinacea* explants inoculated on rooting medium (MS + NAA). Arrow heads at 10 days and 45 days indicate the appearance of root and flower primordia respectively. Data represent mean ± SE of three experiments.

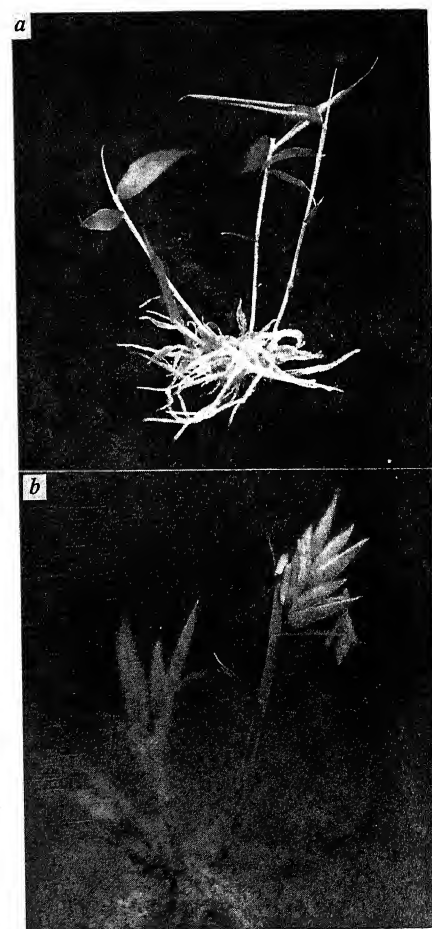


Figure 2. Plantlets of *Bambusa arundinacea* showing (a) adventitious roots (10-day-old), (b) precocious flowers with stamens (45-day-old).

literature, establishing a relationship between peroxidase activity and various stages of organogenesis. However, Rama Rao *et al.*⁵ have shown that the peroxidase activity remains minimum at fiber initiation and elongation and becomes maximum at fiber maturation in cotton.

Further, our observation that the formation of *in vitro* roots and flowers precedes the minimum level of peroxidase activity is in agreement with that on *in vitro* rhizogenesis in several plant species⁷ and cyclic bud formation in date palm⁸. However, the present data do not support Gaspar's assumption^{2,3}. It seems likely that the *de novo* organogenesis precedes a circumstantial minimum level of peroxidase activity.

In the present study, the multiple shoots of *B. arundinacea* exhibited the *in vitro* precocious flowering when they were maintained on rooting medium (MS medium + NAA) for a long period of 45 days. These results indirectly confirm those of Nadgauda *et al.*⁹, who reported the *in vitro* precocious flowering in this species on cytokinin-enriched multiplication medium. It may be possible that multiple shoots of the seedling line used in the present study possessed the genetic potential for precocious flowering which got expressed on accumulation of

sufficient cytokinin (BA) level due to undergoing several cycles on the multiplication medium. Therefore, the *in vitro* precocious flowering in bamboos as reported in the present study and elsewhere⁹⁻¹¹ seems to be independent of culture conditions (light, medium, temperature, etc.), and cytokinin (BA) probably helps only in expression of this potential.

Hence, we conclude that the low peroxidase activity reflects early events (e.g. cell division and elongation) in the sequence of *de novo* organogenesis rather than organ-specific differentiation of root or flower. Further, the *in vitro* precocious flowering in bamboos is probably genetically controlled, with cytokinin (BA) playing a complementary role in the process.

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INDIAN ACADEMY OF SCIENCES

Discussion Meeting on Instability and Transition in Fluid Flows

22-25 November 1996 - Orange County, Coorg District, Karnataka

This discussion meeting is intended to get together Indian scientists active in the area to discuss current research as well as possible future directions, and to encourage formulation of any joint projects that might be of interest to them. Speakers at the meeting will include Indian scientists pursuing research in stability and transition as well as some visitors from abroad, including Dr Jitesh Gajjar (University of Manchester), Prof. Michael Gaster (Queen Mary & Westfield College, London) and Prof. K. R. Sreenivasan (Yale University). A small number of places in the meeting will be available for students, university faculty and other scientists either working in the area or seriously intending to do so. If you are interested in attending the meeting, please send a copy of your bio-data with a brief statement of interest to

Prof. Roddam Narasimha
Centre for Atmospheric Sciences
Indian Institute of Science
Bangalore 560 012

(Fax: 080-334 1683, e-mail: roddam@cas.iisc.ernet.in).

Participants will be required to be present in Bangalore before the evening of 21 November 1996 and will be brought back to Bangalore on the evening of 25 November. Arrangements for transport to and accommodation at the venue of the meeting will be taken care of by the Indian Academy of Sciences.

Gene therapy: Principles, practice, problems and prospects

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The remarkable advances made in recombinant DNA technology over the last two decades have paved way for the use of gene transfer to treat human diseases. Several protocols have been developed for the introduction and expression of genes in humans, but the clinical efficacy has not been conclusively demonstrated in any of them. The eventual success of gene therapy for genetic and acquired disorders depends on the development of better gene transfer vectors for sustained, long term expression of foreign genes as well as a better understanding of the pathophysiology of human diseases. It is heartening to note that some of the gene therapy protocols have found other applications such as the genetic immunization or DNA vaccines, which is being heralded as the third vaccine revolution. Gene therapy is yet to become a dream come true, but the light is seen at the end of the tunnel.

THE advent of recombinant DNA technology and the availability of a wide array of gene cloning techniques have had a dramatic impact on the conduct of research in biology and medicine. The molecular biological revolution has opened the floodgates for basic and applied research and researchers are using genes as double-edged weapons for both understanding basic cellular processes as well as biotechnological applications. Techniques such as polymerase chain reaction, non-radioactive nucleic acid probes, ribozymes, transgenics, gene knockouts, chromosome jumping and fluorescent *in situ* hybridization have revolutionized disease diagnosis and therapy. Genetic information in the form of expressed sequence tags (ESTs) and whole genome sequences are filling computer data banks faster than ever and never has gene cloning found more prominence in biology than it has now¹. Thanks to the brute force approach of positional cloning, it is now possible to identify a disease gene without any knowledge of its biochemical function. The difference between basic and applied research is disappearing fast and the results of basic research are finding immediate biotechnological applications. Gene cloning has enabled us to link DNA repair and cancer, transcription and xeroderma pigmentosum, cell cycle and malignancy and glycolysis and Huntington's disease. Thus, genes are the ultimate molecular switches that control various cellular processes and

abnormal gene expression can manifest in the form of specific genetic disorders and even compromise the ability of an individual to destroy invading pathogens and tumour cells. It is this overwhelming evidence in favour of genes and their critical role in maintaining physiological homeostasis that has strengthened the notion of using genes as therapeutic agents. The idea of delivering genes into humans to correct a disease phenotype remained a biologist's fantasy for a long time until the last decade, when it has been accepted as a scientifically viable proposition, recognized as an independent discipline and christened 'gene therapy'.

Principles and practice

The ultimate goal of human gene therapy is to replace a defective gene with a normal one via targeted insertion into the genome by homologous recombination. Such a strategy, referred to as gene replacement therapy, would not only permit physiological regulation of the transgene but also eliminate the possibility of insertional inactivation of other cellular genes, which happens during random integration of the foreign gene. However, the frequency of random integration versus site-specific integration is so overwhelmingly in favour of the former that gene replacement therapy continues to elude biologists. Recent studies with adeno-associated viral vectors have demonstrated that it is possible to deliver genes to a specific locus in human chromosome 19 and this is an improvement over random integration of genes². A major advantage of integrating foreign genes into the genome is that the expression is relatively stable and long-lasting and in case of dividing cells, the gene is acquired by the daughter cells as well. An alternative approach has been to avoid genome integration altogether and express genes transiently. However, in this case, the foreign DNA is more susceptible to nuclease degradation, and the gene expression is not as stable as seen in integrated genes. Transient vector systems are appropriate for therapies requiring short-term expression, such as in cancer therapy protocols but is not favoured for long-term correction of genetic defects. Overall, the current strategy for gene therapy largely centres around gene augmentation therapy, wherein the foreign gene

replaces the product of the defective or missing gene, but does not physically replace the defective gene itself. At present, two gene transfer strategies are in vogue: the *in vivo* approach, which involves introducing genes directly into the target organs of an individual whereas in the alternative *ex vivo* approach, cells are isolated for gene transfer *in vitro*, followed by transplantation of genetically modified cells back into the patients³.

All the genetic manipulations that can be performed in humans can be classified into four distinct categories: i) somatic gene therapy (correction of a genetic defect in the somatic cells of the body), ii) germ-line gene therapy (introduction of genes into the germ cells for the correction of the genetic defect in the offsprings), iii) enhancement genetic engineering (gene transfer for improvement of a specific trait, such as introducing a growth hormone gene to increase height), iv) eugenic genetic engineering (genes may be inserted to alter or improve complex traits such as intelligence and personality). While somatic gene therapy is now being attempted on human patients, germ-line gene therapy and enhancement genetic engineering are being carried out in laboratory and farm animals. Germ-line gene therapy is not

being attempted in humans for reasons that are as much ethical as technical and eugenic gene therapy is far beyond our technical capabilities and ethically questionable. In fact, the technical problems associated with somatic gene therapy in humans are so overwhelming, that unless we gain sufficient expertise in performing successfully gene transfer in the somatic cells, it may not be possible to carry out other genetic manipulations in humans⁴.

Somatic gene therapy was initially formulated for the treatment of monogenic defects, but now holds promise for a wide range of disorders including cancer, neurological disorders, heart disease and infectious diseases (Table 1). A variety of gene delivery strategies have been developed in the last decade for the treatment of human diseases and these can be grouped in two major categories: the viral and non-viral methods. Both these approaches are being pursued vigorously, although gene transfer through the viral vectors is more efficient than that by non-viral methods. Over several million years, viruses have evolved efficient mechanisms for the delivery of their own genomes into cells they infect and the strategy has been to replace these genes with those

Table 1. Genetic and acquired diseases amenable to gene therapy*

Disease	Therapeutic gene	Strategy	Vector	Target cells/tissue
Genetic disorders				
Cystic fibrosis	CFTR	<i>In vivo</i>	Adenovirus	Nasal epithelium
		<i>In vivo</i>	Cationic lipids	Nasal epithelium
Familial hypercholesterolaemia	LDL receptor	<i>Ex vivo</i>	Retrovirus	Hepatocytes
SCID	ADA	<i>Ex vivo</i>	Retrovirus	T cells, CD34 ⁺ stem cells
Haemophilia	Factor VIII/Factor IX	<i>In vivo</i>	Retrovirus	Hepatocytes, skin, muscle
		<i>Ex vivo</i>	Retrovirus	Hepatocytes, myoblasts
DMD	Dystrophin	<i>In vivo</i>	Retrovirus	Skeletal muscle
		<i>Ex vivo</i>	Retrovirus	Myoblasts
Acquired disorders				
Cancer	Interleukins, HSV-TK, TNF, HLA-B4, tumour suppressor genes	<i>Ex vivo</i>	Retrovirus	Tumour cells
		<i>In vivo</i>	Cationic lipids	Tumour cells
Cardiovascular disorders	tPA	<i>In vivo</i>	Cationic lipids	Endothelial cells
			Adenovirus	Endothelial cells
Alzheimer's disease	NGF	<i>Ex vivo</i>	Retrovirus	Fibroblasts
		<i>In vivo</i>	Adenovirus	Neuronal cells
Parkinson's disease	TH	<i>Ex vivo</i>	Retrovirus	Fibroblasts
			Adenovirus	Neuronal cells
AIDS	HSV-TK, HIV antigen, RevM10	<i>Ex vivo</i>	Retrovirus	T cells
	Cytokine			Hepatocytes
				Hematopoietic stem cells

Abbreviations: CFTR, Cystic fibrosis transmembrane regulator; SCID, severe combined immunodeficiency syndrome; DMD, Duchenne muscular dystrophy; ADA, adenosine deaminase; HSV-TK, herpes simplex virus thymidine kinase; NGF, nerve growth factor; TH, tyrosine hydroxylase; LDL, low density lipoprotein; tPA, tissue plasminogen activator.

*This list is not exhaustive.

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of therapeutic interest such that the virus can only infect and deliver the therapeutic gene to the host but cannot replicate or cause disease. Of the various viruses that are being manipulated this way, research on retroviruses, adenoviruses and adeno-associated viruses has progressed very rapidly and deserves to be discussed in greater detail.

Viral vectors

Retroviruses as gene transfer vehicles have gained prominence because intense research on these viruses in the last two decades has generated a great deal of information on the biology and safe handling of these viruses. The work-horse for retroviral gene therapy has been the murine leukemia virus (MLV), although efforts are under way to develop HIV-based vectors so that even non-dividing cells can be infected. The first step in the development of a replication-defective recombinant retroviral vector involves the replacement of viral structural genes such as *gag*, *pol* and *env* by the therapeutic gene of interest. This vector is then transfected into a packaging cell line that provides the viral structural proteins *in trans*, so that the recombinant retroviral genome, by virtue of its packaging signal (ψ), is packaged and replication-defective retroviruses are generated. When such viruses infect the host cells, the recombinant retroviral RNA is reverse transcribed and integrated randomly into the host genome. In the absence of viral genes, the therapeutic gene is transcribed from the viral LTRs or in some cases from an internal promoter and the protein of interest is synthesized (Figure 1). Depending on the envelope protein produced in the packaging cell lines, one can generate either ecotropic viruses (which can infect rodent cells) or amphotropic viruses (capable of infecting other species including human)^{5,6}. Retroviruses can infect any cell type and in order to impart specificity, the wild type envelope proteins are being modified to include proteins that can dock with specific cell surface receptors, so that they can be targeted to specific cell types. For example, an ecotropic virus which cannot infect human cells can be engineered to infect human cells expressing erythropoietin receptor, by replacing 150 amino acids of the virus envelope protein with that of erythropoietin⁷. In a different approach, the viral envelope protein was converted into an asialoglycoprotein by coupling chemically to lactose and such a virus was able to infect specifically hepatocytes which contain the asialoglycoprotein receptors. Transgene expression can also be made tissue-specific by using internal promoters that function only in specific tissues. Another improvement involves the inclusion of internal ribosome entry sites to promote cap-independent translation initiation at internal initiation codons so that multiple proteins can be expressed from a single poly-

cistronic mRNA⁶. Several steps have been taken to prevent the generation of wild type virus such as expression of structural genes in the packaging cells under different eukaryotic promoters, mutation of the residual AUG codon in the recombinant vector, etc., so that even multiple recombination events are unlikely to generate wild type retroviral particles. The major concern of retroviral gene transfer is the insertional mutagenesis of growth regulatory genes during retroviral integration which could lead to cancer. Retroviral vectors are used mostly in *ex vivo* gene transfer experiments, although it has been shown that they can infect a regenerating liver when administered intravenously into a hepatectomized animal⁸.

Adenoviruses can infect a wide range of cell types and live adenoviruses have been used as vaccines in US military personnel without any major side effects. Two serotypes, Ad5 and Ad2 have been extensively studied, the genome has been (36 kb DNA) sequenced. Of the several genes encoded by the adenoviral genome, the proteins encoded by the E1 gene are crucial for virus replication and deletion of E1 gene renders the virus replication defective. Therapeutic genes up to 3.2 kb can replace the E1 gene and introduction of the recombinant viral DNA into cells expressing the E1 gene products results in the generation of replication-defective adenoviruses^{9,10}. Genes up to 7.5 kb can be accommodated by deletion of another non-essential gene,

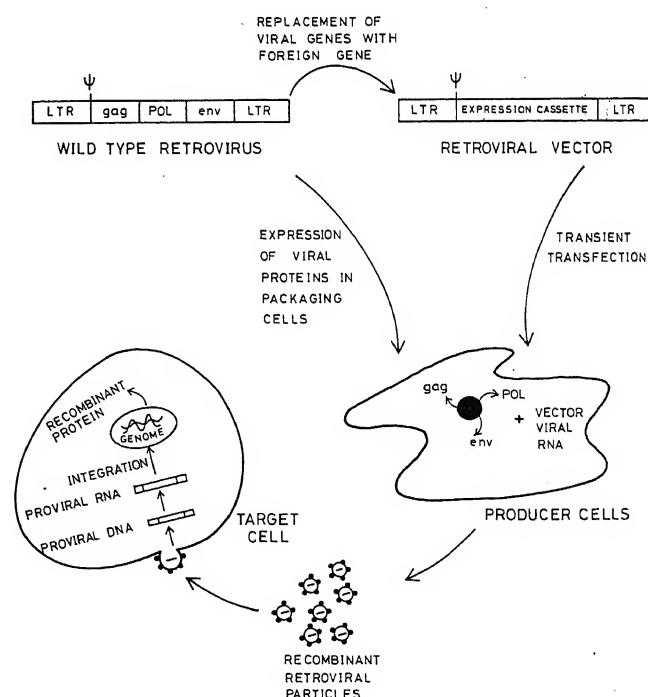


Figure 1. Retroviral gene transfer. Construction of a retroviral vector, production of recombinant retroviruses, transfection of target cells and synthesis of recombinant protein are shown (see text for details).

E3. Adenoviruses are attractive candidates for gene therapy of lung disorders because of their natural tropism for infecting respiratory epithelium¹¹. Unlike retroviruses, they can infect post-mitotic cells as well and this has raised the possibility of their use for gene transfer into brain^{12,13}.

Adeno-associated virus (AAV), a non-pathogenic human parvovirus has aroused a lot of interest as a vector for gene therapy. AAV is a dependovirus and requires a helper virus coinfection for viral replication. In the absence of coinfection with a helper virus such as adenovirus, herpes virus or cytomegalovirus, the viral genome integrates into the human genome usually at a specific site, 19q13.3-qter. The therapeutic gene of interest is cloned between the two inverted terminal repeats or ITRs and the recombinant plasmid is transfected along with another plasmid expressing the viral structural proteins, Rep and Cap into cells infected with a helper virus. The recombinant AAV particles thus generated are purified and used for infection of target cells. The biology of AAV vectors is not as well understood as that of retroviruses or adenoviruses and this vector system is still in its infancy. The potential use of this virus as a gene transfer vector has intensified research on the life cycle of the virus and understanding the mechanism of latency, site-specific integration and development of good packaging cell lines will be the key to the success of AAV as a vector for gene therapy².

Non-viral approaches

Despite the fact the most of the research in gene therapy has focused on the development of viral vectors, the clinical efficacy as well as safety of these methods is still questionable. Further, the technical complexity and cost of performing viral-based gene therapy is another factor that has led to a resurgence of interest in the development of non-viral gene therapy. Unlike the viral vectors, which are used in both *in vivo* and *ex vivo* strategies, majority of the non-viral methods follow the *in vivo* approach, so that a successful non-viral technique can lead to the possibility of using genes directly as drugs. In general, non-viral methods for gene transfer can be categorized as physical and chemical methods. Of the various chemical methods, delivery of genes complexed with ligands for cell surface receptors and cationic lipids have been under intense study. The concept of delivering genes into specific tissues via receptor-mediated gene delivery was examined extensively with the liver asialoglycoprotein receptors. These receptors which are exclusively present on hepatocytes bind to certain glycoproteins lacking the terminal sialic acid. The strategy has been to couple orosomucoid (an asialoglycoprotein) to poly-L-lysine and the conjugate is condensed with plasmid DNA via electrostatic interactions.

The resulting soluble complex when injected intravenously, is internalized into hepatocytes via the asialoglycoprotein receptors and transgene expression was detected up to several weeks¹⁴ (Figure 2). This strategy was later extended to other cell surface receptors such as transferrin receptor, etc.¹⁵. While the receptor-mediated gene delivery is an attractive strategy as it takes advantage of the normal physiological pathway, the major problem has been the degradation of DNA by the lysosomal enzymes in the endosomes. Co-administration of lysosomotropic agents such as chloroquine has been shown to enhance gene expression. Since adenoviruses escape lysosomal degradation by acidification of endosomes, several strategies have been designed to develop adenovirus-linked molecular conjugate vectors. For example, monoclonal antibodies against a heterologous epitope on the hexon protein of the adenovirus envelope is covalently linked to polylysine which was then coupled to plasmid DNA. Such a conjugate can now interact with adenoviruses expressing the epitope on the envelope and delivery of such molecular conjugates has been shown to facilitate more efficient gene transfer than that by the conventional receptor-mediated gene delivery techniques¹⁶. In their present form, molecular conjugate vectors have several applications for *in vitro* use, but use in somatic gene therapy awaits more improvements in the vector design and delivery.

The observation that positively charged liposomes or cationic lipids can entrap negatively charged DNA has led to the possibility of their use as gene transfer vehicles *in vivo*. Several cationic lipid formulations are now being used for transfection of cells in culture and

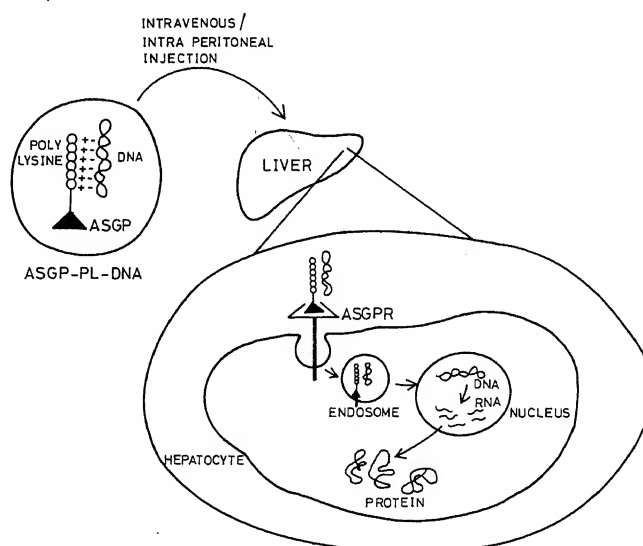


Figure 2. Receptor-mediated gene delivery. The delivery of asialoglycoprotein-poly-L-lysine-DNA complex (ASGP-PL-DNA) into hepatocytes, its internalization into hepatocytes via the asialoglycoprotein receptor (ASGPR), delivery of DNA into the nucleus and synthesis of recombinant protein are depicted.

some of these have been shown to promote gene delivery into a variety of organs *in vivo* as well^{17,18}. Two potential applications of cationic lipids are the aerosol delivery of genes to lungs and in the development of cancer vaccines. The cationic lipid-mediated gene delivery to lung has advantages over adenovirus-mediated gene transfer in that, it can be administered as an aerosol and is non-immunogenic. Intratumoral injection of DNA-lipid complexes is being attempted to evoke specific immune response against a given tumour type by expression of heterologous antigens, cytokines and co-stimulatory molecules^{19,20}.

Amongst the physical methods of gene transfer, direct DNA injection into skeletal muscle has created a lot of excitement and has led to the possibility of using genes as vaccines. Several researchers had attempted direct gene transfer since the early 1960s, but these studies were not taken seriously due to inefficient gene transfer and low levels of expression. It was in the year 1990, Wolff *et al.*²¹ reported that direct injection of plasmid DNA into skeletal muscle results in sustained expression of foreign genes but the level of expression is too low to derive a therapeutic benefit for the treatment of genetic disorders. However, researchers at Vical and Merck pursued these studies further and demonstrated that the low levels of foreign proteins synthesized in the host cells were processed along the Class I MHC pathway, leading to presentation of peptides on cell surface resulting in a potent immune response against the foreign antigens and thus was born the concept of DNA vaccine or genetic immunization. These researchers demonstrated that immunization by direct injection of plasmid DNA encoding influenza nucleoprotein protects mice from a lethal challenge of influenza virus²². In the last few years, these results were confirmed with a variety of genes encoding several viral and parasite antigens and many of these studies have now been approved for clinical trials in the United States. Direct injection of plasmid DNA encoding foreign antigens into skin has also been shown to evoke a protective immune response. Another physical method of gene transfer that is becoming popular is the delivery of DNA using 'gene gun'. This method, developed originally for transformation of plants, involves delivery of genes by bombardment with gene-coated gold particles into tissues such as liver, skin, mammary gland and muscle. Direct gene delivery is now being used to map promoter elements that regulate gene expression *in vivo*, in transfection of herpes simplex thymidine kinase genes into melanomas to render them sensitive to gancyclovir and as a vaccination strategy²³⁻²⁶. Thus, direct gene transfer is one example of a technique that failed to make an impression in the treatment of genetic disorders but made an enormous impact in the field of vaccine development and is being heralded as the third vaccine

revolution. Other physical methods such as electroporation and microinjection have only limited scope in somatic cell gene therapy and their use is largely restricted to transfection of cells in culture and transgenic animal research.

The success of gene therapy depends not only on the gene delivery mechanism but also on the choice of target tissues. Cell types which can grow and divide *in vitro*, such as keratinocytes, myoblasts, hepatocytes, endothelial cells and hematopoietic stem cells, are amenable for both *ex vivo* and *in vivo* gene delivery strategies whereas *in vivo* methods are preferred for cell types such as neuronal cells which are not easily amenable for *ex vivo* manipulations. The choice of tissue also depends on the function of the gene product. In case of diseases such as hemophilia, the gene can be delivered and expressed in any tissue, provided that the protein is released into the blood stream. In case of diseases such as cystic fibrosis and Duchenne's muscular dystrophy, the gene should be delivered to the specific cell types where the gene function is deficient, in order to derive the therapeutic benefit. Further, the ease and efficiency with which the transfected cells can repopulate the affected tissue is another criterion that poses limitations on *ex vivo* gene therapy. One of the main targets for *ex vivo* gene therapy is the hematopoietic stem cells and retroviral gene transfer has been the most preferred method of gene transfer into these cells. In case of gene therapy for neurological disorders, gene transfer is being attempted using adenoviral and herpes viral vectors. While *ex vivo* gene transfer is usually performed with autologous cells, heterologous cells or even immortalized cells placed in immuno-isolation devices have been used, although the potential safety concerns make them less attractive for use in humans²⁷. Antisense gene transfer is another attractive strategy for the treatment of disorders such as β -thalassemia, in which accumulation of α -globin chains in red blood cells results in their premature destruction. For example, infection of K562 erythro-leukemia cells with AAV expressing human α -globin gene in antisense orientation results in significant inhibition of the endogenous α -globin gene²⁸.

The potential of a gene delivery system is first ascertained in cultured cells or in animal models, using reporter genes such as luciferase, growth hormone, β -galactosidase, etc. Based on these studies, pre-clinical trials are conducted in animal models to evaluate the levels and duration of expression of specific therapeutic genes. The clinical efficacy of the gene therapy procedure is then evaluated in human patients. Prior permission from specific regulatory agencies is necessary for performing gene therapy in humans. The Recombinant DNA Advisory Committee (RAC) and the Food and Drug Administration (FDA) in the United States and the Gene Therapy Advisory Committee (GTAC) in the United

Kingdom are empowered to authorize the conduct of such clinical trials in these countries. Several other countries such as The Netherlands, Italy, Germany, France, China, Japan, Switzerland, Sweden and Austria have also evolved guidelines for gene therapy research and initiated clinical trials for human gene transfer. These guidelines are being formulated in India as well.

Problems and prospects

The viral and non-viral gene delivery strategies have been fairly successful in cell culture systems and animal models but the therapeutic success of clinical trials in humans still remains questionable. Two types of clinical trials are being conducted in humans using these vectors: gene marking and therapeutic studies. In gene marking experiments, human cells which have a potential therapeutic use, such as hematopoietic stem cells, T cells, tumour-infiltrating lymphocytes, malignant cells, etc., are removed from the patient, cultured and transfected with retroviral vectors encoding marker genes and re-introduced into the patient from whom the cells are removed. At different periods after re-introduction, cells are recovered and the presence of marker gene or its products is examined. Such studies have demonstrated that gene transfer is feasible in humans and depending on the vector system used, marker gene expression could be detected for a period ranging from a few days up to three years²⁹⁻³¹. Cell-marking studies have also been used to detect residual cancer cells in the marrow infused into patients during autologous bone marrow transplantation, for treatment of diseases such as leukemia²⁹. Thus, cell-marking studies have been very useful in demonstrating the feasibility and safety of human gene transfer, especially using the retroviral vectors. On the contrary, the therapeutic trials are aimed at transfer of therapeutic genes into patients for treatment of specific genetic disorders and cancer. Several therapeutic clinical trials have been initiated in the last few years and the results of some of these are now available. Some examples of successful *ex vivo* human gene transfer studies are: transfer of adenosine deaminase (ADA) gene transfected T cells or cord blood cells to children suffering from adenosine deaminase deficiency leading to severe combined immunodeficiency (SCID)³²⁻³⁴, transfer of autologous cells encoding cytokine genes into cancer patients, transfer of autologous hepatocytes encoding low-density lipoprotein (LDL) receptor for individuals with familial hypercholesterolaemia³⁵, intracerebral transfer of fibroblasts expressing herpes simplex virus thymidine kinase gene for the ablation of brain tumours³¹, etc. Examples for successful *in vivo* gene transfer are: delivery and expression of the gene encoding cystic fibrosis transmembrane conductance regulator (CFTR) in the pulmonary epithelium of cystic fibrosis patients using

adenoviral vectors and cationic lipids^{36,37}; human leukocyte antigen (HLA-B7) and β 2-microglobulin gene transfer in tumours by cationic lipid-mediated gene transfer^{38,39}, etc. In many of these clinical trials, patients continued to receive conventional drug and/or protein replacement therapies along with gene therapy leading to difficulty in the interpretation of results. These problems are discussed below with specific examples:

In the year 1990, a clinical trial was initiated in two children suffering from ADA deficiency, using an *ex vivo* retroviral gene transfer protocol involving autologous T cells isolated from these patients³⁴. Prior to gene therapy, these children were undergoing enzyme replacement therapy wherein bovine ADA conjugated with polyethylene glycol (PEG-ADA) was being administered at regular intervals for 2-4 years. In the gene therapy approach, T cells were isolated from these children, induced to proliferate *in vitro* and then transfected with a retroviral vector encoding the normal ADA cDNA. Such genetically modified lymphocytes were expanded in the laboratory and periodically infused into the autologous patients for two years and the effect of the therapy was evaluated for a period of five years. However, enzyme replacement therapy was also continued during this period and several clinical parameters such as increase in T cell numbers, ADA levels in the circulating T cells, T cell cytokine release, cytotoxic T cell activity and skin test response to common antigens were evaluated over a period of five years. Comparison of these parameters with those observed prior to the commencement of gene therapy indicated a definitive improvement, although the continued enzyme infusions during this period makes it difficult to evaluate the benefit derived from gene therapy alone. The problem with this clinical trial has been the variation in the level of ADA⁺ circulating T cells seen in these two patients, which varied from 0.1 to 60%. Nevertheless, this study demonstrated that *ex vivo* retroviral gene transfer is a feasible and safe strategy for the treatment of ADA deficiency and several protocols, each using different strategies involving different retroviral vectors targeted to different cell types have been approved for clinical trials and results of these studies are still awaited³⁹.

Familial hypercholesterolaemia is another genetic disorder that was the target for *ex vivo* retroviral gene therapy for a long time. This genetic defect, resulting in deficiency of LDL receptors in the liver, results in high levels of serum cholesterol and LDL cholesterol, leading to premature atherosclerosis and myocardial infarction. The gene therapy protocol for the correction of this disorder involves isolation of hepatocytes from the patient by partial hepatectomy, *in vitro* transfection with retroviral vectors carrying a normal LDL receptor gene and reintroduction of these genetically modified hepatocytes into the portal circulation of the patient.

Studies were first carried out in rabbits, later in dogs and baboons and long term expression (up to 1.5 years in baboons) of the foreign gene was demonstrated in these animals. Based on these results, a clinical trial was initiated in a 28-year-old French Canadian woman on 5 June 1992, suffering from this genetic disorder³⁴. Evaluation of molecular and metabolic data over a period of 18 months after gene transfer suggested that the genetically modified hepatocytes had engrafted stably in this patient, recombinant gene expression could be detected throughout this period and there was a significant reduction in LDL cholesterol and LDL/HDL ratio. However, similar to the ADA-deficiency studies, this study was also compromised by the fact that other conventional therapies were being administered during this period. Despite this drawback, the study demonstrated that liver-directed *ex vivo* retroviral human gene therapy is a feasible and safe approach and can be applied for the treatment of other hepatic genetic disorders as well.

Of the *in vivo* approaches, introduction of adenoviral vectors encoding the normal CFTR to the nasal or bronchial epithelium of patients with cystic fibrosis has been shown to result in the expression of CFTR in the lung epithelium^{36,37}. CFTR cDNA containing plasmids have been directly administered to the nasal epithelium of these patients via cationic lipid-mediated gene transfer. In all these clinical trials, evidence for transfer of normal CFTR gene into the respiratory epithelium was clearly established, however, expression is observed in only about 5% of the target cells³¹. Further, the clinical efficacy, assessed by the correction of abnormal potential difference across the nasal epithelium, is observed in only some of the patients who have undergone gene transfer. Another problem associated with adenoviral gene transfer to the lung is the local and systemic inflammatory response evoked in some patients which was not observed in animal trials³⁹.

In addition to the treatment of genetic disorders, the gene transfer techniques have also been applied extensively in the treatment of cancer and other acquired disorders. The cancer gene therapy strategies are of two types: correction of abnormal genotype of the tumour cells or destruction of tumour cells. The corrective gene therapy is aimed at introducing wild type tumour suppressor genes or genes encoding dominant negative mutants of oncogenes that are mutated in the tumour cells⁴¹. The major limitation of this approach has been the difficulty in delivering the corrective gene into every tumour cell. Thus, efforts are largely centered on the alternative approach aimed at destruction of the abnormal tumour cells by expression of genes encoding cytokines or toxins. Autologous tumour cells are modified *ex vivo* by the transfer of genes encoding cytokines, allogenic MHC molecules or heterologous antigens to enhance their immunogenicity⁴². Another strategy has been to

introduce genes encoding cytokines or tumour necrosis factor into tumour-infiltrating lymphocytes (TILs) and their autologous transfer into cancer patients⁴³. In the cytotoxic gene therapy approach, genes encoding enzymes such as herpes simplex thymidine kinase, cytosine deaminase, etc., are cloned downstream of tumour-specific promoters and introduced into tumour cells. These enzymes convert pro-drugs into toxins that destroy the tumour cells. Surprisingly, even non-transfected neighbouring cells are destroyed and this bystander effect is probably due to the transfer of the toxins between cells via cell-junctions^{41,44}. Despite promising results obtained in animals, there is no convincing evidence that any of the gene therapy protocols actually led to regression of tumours in humans. Thus, the problem of inconclusive and inconsistent results obtained from clinical trials of gene therapy for genetic disorders is also true for that of cancer⁴⁵.

In the final analysis, it is clear that the move from mouse to man is not going to be easy. The conduct of clinical trials has been a great education and we now have a fair knowledge of the actual problems and pitfalls of the various gene delivery strategies. None of the clinical trials conducted so far could convincingly demonstrate the therapeutic benefit of gene therapy and has not been as effective as expected⁴⁶⁻⁴⁸. This is in sharp contrast to the results obtained in animal experiments which raised the expectation of gene therapy so much that the potential of gene transfer strategies became greatly exaggerated and mere conduct of a clinical trial led to a steep rise in the stock market prices of companies involved in gene therapy research. While nobody debates the importance and long term potential of human gene therapy, it is now clear that the current gene transfer techniques are largely inadequate to promote sustained, high level expression of foreign genes *in vivo*. Thus, in order for gene therapy to become a reality, it is essential that more efforts are directed towards understanding the pathophysiology of diseases as well as the mechanism of gene regulation *in vivo*. More basic research should be initiated into vector development and the role of chromatin, enhancers and locus-control regions in the regulation of gene expression *in vivo* has to be investigated in greater detail. Despite the various setbacks, gene therapy has witnessed a rapid growth in the last decade and fundamental advances made in molecular and cell biology will continue to contribute to the progress of gene therapy. For example, a recent study on hereditary tyrosinemia type I indicates that as few as 1000 transplanted hepatocytes can repopulate almost an entire liver, suggesting that retrovirally transfused hepatocytes can be induced to engraft into host liver and proliferate extensively, when appropriate selective pressures are applied⁴⁹. Such studies could solve the problem of low efficiency of gene transfer and bring

ex vivo hepatic gene therapy much closer to reality. Gene therapy is yet to become a dream come true, but the light is seen at the end of the tunnel!

Need for gene therapy studies in India

With the explosive increase in the availability of information on human genome, several genetic disorders would become candidates for gene therapy. As already indicated, besides genetic diseases, gene therapy has the potential for treatment of several other disorders such as cancer, cardiovascular and neurological disorders and infectious diseases. At the same time, the initial hype and euphoria are giving place to a realistic assessment of the basic information that is needed to make this exciting approach a success. This 'back to the basics' approach lends tremendous scope to initiate fundamental studies on (i) design of newer vectors for gene delivery, (ii) newer approaches to systemic delivery, (iii) targeting to specific tissues and cells, (iv) stability and duration of expression of the gene introduced, (v) The status of the introduced gene *in vivo* – integrated into the chromosome or episomal?, (vi) design of appropriate animal models, (vii) assessment of risk-benefit status, and (viii) an understanding of the molecular basis of cellular humoral immune response in case of DNA vaccines.

At the present stage, only 3 or 4 laboratories have initiated research in this area in the country. At Delhi University (South Campus), delivery of macromolecules such as toxins, drugs and nucleic acids into viable cultured cells through the use of reconstituted sendai virus envelopes (RSVE or virosomes) has been demonstrated^{50,51}. Since this involves fusion and delivery of genes into the cytoplasm, receptor-mediated delivery into lysosome and consequent degradation in the endosomes is circumvented. This approach needs to be extrapolated under *in vivo* conditions. At the Cancer Research Centre Bombay an approach to treat oral cancer through gene targeting to oral mucosal cells is under examination. In our laboratory, receptor-mediated targeting and expression of growth hormone gene in rat liver have been demonstrated with the use of a regulatable promoter (cytochrome P-450 promoter induced by phenobarbital)⁵². Further, newer approaches to delivering DNA to hepatocytes by perfusion of liver with DNA-lipofectin complex have been demonstrated⁵³. More recent studies have involved a comparative study of the efficiency of different carriers and routes of injection on the uptake and expression of reporter genes in experimental animals both from the perspective of gene therapy and DNA vaccination. Non-viral gene delivery strategies are being examined for their therapeutic and vaccination potential using expression vectors encoding growth hormone and antigens of rinderpest virus. There is scope to extend these genetic immunization studies to other models in-

volving Japanese encephalitis virus, malarial parasite, etc.

The need of the hour is to initiate more studies in different systems on the various aspects mentioned earlier. The field is still at its infancy and relevant. For the first time in the history of mankind, a rational approach to actually treating a genetic disorder has become at least an experimental feasibility. A welcome fallout has been the potential of this approach to treat cancers in particular. Recognizing the importance and relevance of this approach, the Department of Biotechnology has constituted a group of experts to activate this area of research and invite proposals. It is to be hoped that the scientific community in the country would come forward to work in this area of biomedical research that lends scope for collaboration between medical and basic research scientists.

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Micelles: Self-organized surfactant assemblies

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Surfactants can self-organize under specific environmental conditions in solution to form 'micelles'. The micelles are of two types, 'normal micelles' (called micelles), which are formed by surfactant association in water or polar solvents, and 'reverse micelles', which are formed in nonpolar media. The surfactants and micellar solutions have versatile uses in the fields of chemistry, biochemistry, pharmacy, medicine and industry to augment and control solubilization, stabilization, dispersion, cleaning, rates of chemical reactions, enhanced oil recovery, etc. In this review, fundamentals of formation of micelles, their physico-chemical properties and probable uses are presented for an overall grasp of the topic of contemporary interest.

AMPHIPHILES are chemical compounds having dual affinity for water and oil. They have distinct nonpolar (lipophilic or hydrophobic) and polar (hydrophilic or liophobic) sections in their molecules. Soaps, detergents, long chain alcohols (amines, aldehydes, etc.) and lipids constitute the class of amphiphiles. They are surface active, can reduce surface tension of the medium or interfacial tension between two immiscible liquids (e.g. oil and water), can assist solubilization, cleaning, dispersion, emulsification, etc. The amphiphiles comprising soaps and detergents show a special property in solution manifesting characteristic self-organization or association

called 'micelle' formation^{1,2}. Under appropriate conditions, amphiphiles (mostly of lipid types) may also form 'liquid crystals' in solution. The amphiphiles that form micelles and can be potentially used for surface chemical works are termed SURFACE ACTIVE AGENTS or SURFACTANTS. Soaps and detergents come under this heading. Soaps are prepared by the hydrolysis (saponification) of naturally occurring fats and oils, and detergents are synthetically prepared. Big industries are busy with the production of soaps and detergents all over the world. The innumerable possibilities of such products through chemical modification and synthesis and great many uses of amphiphiles in pharmaceutical, chemical, biochemical (including biomedical) and industrial fields have added tremendous scope of research and study in this area of surface and colloid science and technology. The investigations on micelles comprise a significant share in this area, a comprehensive accounting of which is of contemporary interest. In the following sections, the overall state of the art of basic aspects of surfactants and micelles including their potential uses are presented.

Types of micelle-forming amphiphiles or surfactants

The micelle-forming amphiphiles or surfactants essentially fall in two categories, 'ionic' and 'nonionic'. They

basically contain nonpolar polymethylene chain (called the tail) and an ionic or polar group (called the head). The nonpolar tail may have substitution and they may contain phenyl rings; the polar or the ionic head group may have distinctive variations. Table 1 gives examples of several typical micelle-forming amphiphiles whose solution properties have been extensively studied and have potential for future investigative inclusions.

The examples given in Table 1 are only a few of the vast number of surfactants possible directly from natural sources and through synthesis³. It is imperative that numerous possibilities of formation, by way of synthesis, exist.

Requirements for micelle formation

Molecular factor

As stated above, all amphiphile molecules have a distinct hydrophobic (HP) tail and a hydrophylic (HF) head (Figure 1).

Table 1. Examples of typical ionic and nonionic micelle forming amphiphiles^a

Ionics	Nonionics
Sodium palmitate	Polyethylene glycol tertoctyl phenyl ether (Triton $\times 100$)
Sodium oleate	Sorbitan monolaurate (Span 20)
Sodium decyl sulphate (NaDes)	Sorbitan monopalmitate (Span 40)
Sodium dodecyl sulphate (SDS)	Sorbitan monostearate (Span 60)
Sodium dodecyl sulphonate	Sorbitan monooleate (Span 80)
Sodium dodecyl benzene sulphonate (SDBS)	Polyoxyethylene (4) laurylether (Brij 30)
Sodium cholate (NaC)	Polyoxyethylene (23) laurylether (Brij 35)
Sodium deoxycholate (NaDC)	Polyoxyethylene (9) palmitylether (Brij 56)
Sodium chenodeoxycholate (NaDC)	Polyoxyethylene (9) stearylether (Brij 76)
Sodium taurochenodeoxy cholate (NaTCDC)	Polyoxyethylene (20) sorbitan monolaurate (Tween 20)
Sodium bis ethyl hexyl sulfo-succinate (AOT)	Polyoxyethylene (20) sorbitan monopalmitate (Tween 40)
Dodecyltrimethyl ammonium bromide (DTAB)	Polyoxyethylene (20) sorbitan monostearate (Tween 60)
Tetradecyltrimethyl ammonium bromide (TTAB)	Polyoxyethylene (20) sorbitan monooleate (Tween 80)
Hexadecyl (or cetyl) trimethyl ammonium bromide (CTAB)	Octylmethyl sulphoxide
Hexadecyl (or cetyl) pyridinium chloride (CPC)	Tetradecyl <i>N</i> betaine

^aFor ionics, abbreviations are given in parenthesis. They are commercial identities for nonionics. The numbers in parentheses correspond to the number of polyoxyethylene groups.

Depending on the molecular structure and type, a balance between the hydrophilicity and hydrophobicity exists in the molecule. This is called hydrophilic-liphophilic-balance or HLB, which is important in categorizing surfactants as emulsifiers, detergents, wetting agents solubilizing agents, micelle-forming types, etc⁴. It is imperative that surfactants having greater hydrophobicity are more surface active and vice-versa. In solution, mutual likings (interaction) between surfactant molecules, solvent (water) molecules, and surfactant and solvent molecules decide the ultimate state. When self-interactions of both surfactants and solvent molecules cannot be compensated by their mutual interactions, the surfactant molecules tend to associate in a regular pattern forming 'association colloids' or 'micelles'⁵. Instead of crowding at the interface, the amphiphiles hide their tails in the micellar interior creating similar (oily) environment, with their hydrophylic heads remaining out in the aqueous medium. It has been observed that micelle-forming amphiphiles in a homologous series need to have eight or more methylene (CH_2) groups in the chain, lower number cannot conveniently augment nonpolar association of the tails to overweigh the head group repulsion and hydrophilicity culminating to micelle formation (Figure 2).

Physical factors

It is imperative that micelle formation becomes easier for surfactants having greater hydrophobicity (or increased chain length) in a homologous series. The other factors that can influence or control the phenomenon are solvent polarity and type, temperature, presence of additives (salts, etc.) and pressure⁵.

The polarity of the medium favours surfactant association. Nonpolar medium offers environment similar to the surfactant tail so that their tendency of self-association is reduced. In a good nonpolar medium, viz. cyclohexane, carbon tetrachloride, hydrocarbons (heptane, octane, decane, etc.), formation of normal micelle as figured above may be totally absent; instead a reverse orientation of the surfactants with tails out and head groups in the

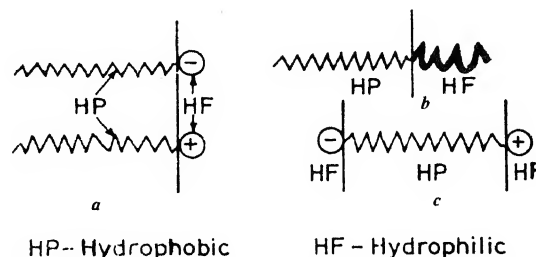


Figure 1 a-c. Perspective representations of surfactants. *a*, ionic; *b*, nonionic; *c*, zwitterionic.

micellar interior may occur; such entities are called 'reverse micelles'. A trace amount of water helps easier and stable formation of reverse micelles. This will be briefly discussed in a subsequent section.

The effect of temperature on micelle formation is essentially guided by the way temperature affects the solubility and other behaviours of surfactants in solution. Normally, surfactant solubility in water does not radically increase with temperature. Desolvation and changed solvent structure play a significant role in this respect. In general, micelle formation is favoured with increase of temperature in the lower range of temperature, at higher temperature range, the formation is disfavoured. The desolvated head groups may end up with greater electrostatic repulsion to resist micelle formation. The situation becomes complex by the changed polarity of the medium at higher temperature. Thus an overall comprehension⁵⁻⁷ of the effect of temperature on the phenomenon of micellization may not be straightforward. At higher temperature, desolvation of the polar head groups of nonionic surfactants leads to phase separation, the solution becomes cloudy. The temperature at which the phenomenon starts is called the 'cloud point'⁵⁻⁸. The temperature stability of nonionic surfactants is often judged by their cloud points.

The surfactants have characteristic temperature-dependent phase behaviours, a knowledge of which in solutions may be profitable for physical-chemical understanding of their solution behaviours. This is shown in Figure 3. In this diagram, there are three distinct zones A, B and C depending on concentration and temperature. In zone A, only surfactant monomers occur in solution. In zone B, monomers remain in equilibrium with micelles, whereas in zone C, monomer and precipitate (or crystals), i.e. a separate phase, exists in solution. This phase map is helpful in the preparation of a solution of a surfactant according to the need of its solution composition. At the point P all the three phases co-exist and by the phase rule, it is an invariant point. The corresponding

temperature is called Krafft temperature (T_K). To avoid or minimize the existence of precipitate, i.e. for the preparation of micellar solution at higher concentration (and only monomer solution at lower concentration), the working temperature should be above Krafft temperature (T_K) (refs 5, 9). The determination of T_K for surfactants is thus important.

The effect of pressure⁷ on self-organization of surfactants has been studied. Pressure initially retards the association and after a threshold value (100–200 MPa), the process is favoured. This is supposed to be a consequence of water structure destruction, by the applied thrust to assist wider distribution of the surfactant molecules in solution to oppose their tendency of association. The release of surfactant monomers from the micelles in the lower range of pressure and their association at higher pressure together with the changed dielectric constant of the solution by the application of pressure also play their specific roles in surfactant organization. This has been supported by the measurement of aggregation number which shows a minimum for ionic surfactants and a rapid initial decrease for nonionic surfactants with respect to pressure.

Additives may have significant effects on surfactant self-organization¹⁰. A salting-out effect of salts influencing the surfactant activity to assist easier aggregation may arise. The study of the influence of salt is important for in most occasions in chemical studies amphiphiles are handled in electrolyte environments. Non electrolytes may both increase and decrease micellization tendency of surfactants^{11,12}. The matter is complex because additives can influence solvent structure (fluidity) and polarity and can undergo direct interaction with the surfactants. In this regard urea and guanidine hydrochloride are conspicuous, they greatly hinder micellization and can break down water structure. The hydrophobic association and water structure destruction have a mutual correlation, a quantitative understanding of the phenomenon is still a matter of further research.

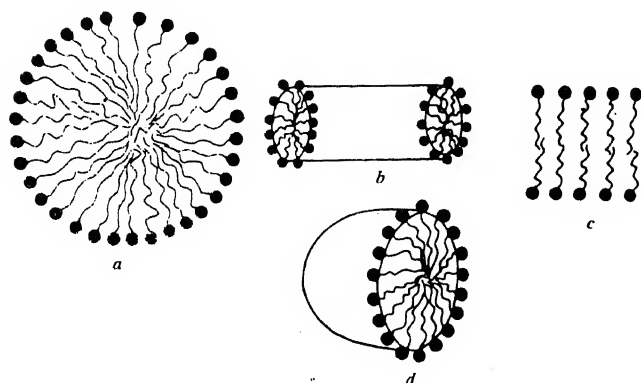


Figure 2 a–d. Schematic representation of geometrical forms of micelles. a, spherical; b, cylindrical; c, lamellar; d, oblate (bisected).

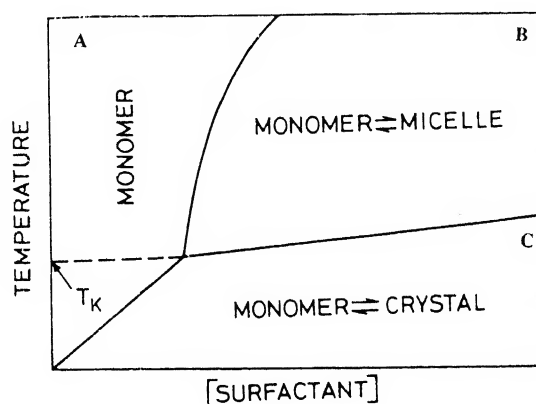


Figure 3. Temperature–[surfactant] profile showing different states in solution and the Krafft temperature, T_K .

Determination of micellar point

The phase map in Figure 3 indicates that under fixed environmental conditions, a threshold concentration is required for the formation of micelle. This threshold or critical concentration is called 'critical micelle concentration' or CMC. Its evaluation (determination) is an important physicochemical exercise for self-assembling surfactant solution.

A good number of methods can be used for the determination of CMC, viz. tensiometry, conductometry, viscometry, light scattering, fluorimetry, calorimetry, spectrophotometry, magnetic resonance, etc. The most frequently used methods are tensiometry, conductometry and fluorimetry, the conductance method is applicable only to ionic surfactants. The physical properties of surfactant solutions measured by different methods at different concentrations demonstrate a distinct feature as shown in Figure 4. The properties show breaks in the plots which are considered as the CMC points for the surfactants under investigation. It is to be noted that the CMC values determined by different methods fall in a narrow range, the CMC is moderately method-dependent. The significant increase in the scattered radiation above CMC in the light scattering method¹³ gives a direct proof of the formation of bigger species by self-association for the scattering phenomenon depends on the size of the scattering units. This and supports by other methods (e.g. self-diffusion by NMR)¹⁴ have established the correctness of the concept of micelles first proposed by McBain¹⁵ of USA (the first Director of the National Chemical Laboratory in independent India) which was severely but wrongly criticized by scientists in a Royal Society meeting in London.

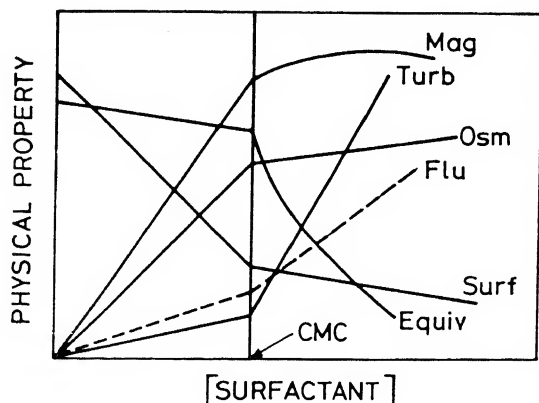


Figure 4. Evaluation of critical micelle concentration by different physical methods. Mag, magnetic resonance; Turb, turbidity; Osm, osmometry; Surf, surface tension; Equiv, equivalent conductance; Flu, fluorescence.

Micellar properties and features

Micelles are surfactant aggregates having regular structures and shapes. From dimension and other considerations they are colloids; their sizes normally cover the range of 1–10 nm. The ionic micelle forms an electrical double layer¹⁶ surrounding it in the interfacial region (Figure 5). By virtue of surface charge and zetal potential, it exhibits electrophoresis under an applied electric field.

The micelles may assume different shapes, the spherical shape is most prevalent; ellipsoidal and cylindrical shapes are also not uncommon^{3,5,16} (Figure 2). The shapes may undergo transition, thus spherical micelles of sodium-dodecylsulphate change to cylindrical configuration in salt environment¹⁷. In presence of salicylates, cetyltrimethyl ammonium bromide, cetylpyridinium chloride, etc. can form long 'worm-like' micelles¹⁸. Such transition greatly influences (increases) the viscosity of the micellar solution, micellar mass increases significantly.

It is tempting to know the number of surfactant monomers that self-organize to form a micelle. The methods of light scattering and fluorescence quenching are conveniently used to estimate the aggregation number^{19,20}. Normally, the aggregation number falls in the range of 20–200; the bile salt micelles can have lower aggregation number²¹ of 4–10. The aggregation number varies with environmental variations. The transition from sphere to rod is associated with significant increase in the aggregation number²².

The presence of ionic groups at the micellar interface causes ion-dipole interaction and water molecules associate to solvate the micelles. The nonionic micelles arrest water molecules at the pallisade layer by hydrogen bonding of water with the polyethylene oxide groups²³. Water may remain trapped in this region. The interfacial properties of micelles are thus influenced by solvation. The solvation can be estimated by various methods, viz.

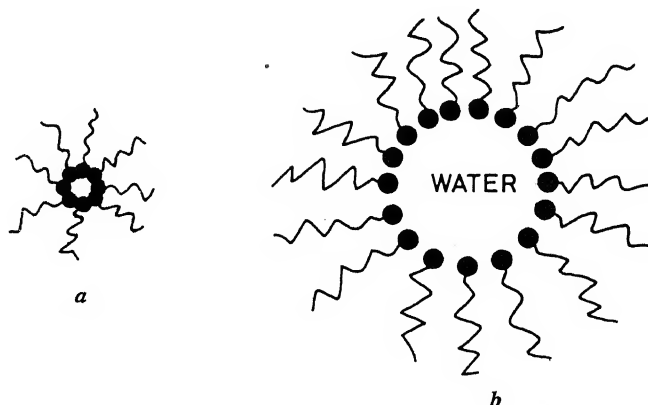


Figure 5a,b. Schematic representation of reverse micelles. a, in absence of water; b, in presence of water.

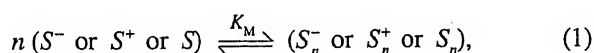
light scattering, IR-spectra, viscosity, electrophoresis, ultrasonic, etc. According to Hartley²⁴, the interior of the micelle is oil-like, the aqueous medium remains outside the interfacial boundary. Menger²⁵ has proposed that water can penetrate inside the micelle up to a certain level, the idea gets support from fluorescence and NMR measurements.

An ionic micelle forms an electrical double layer surrounding its boundary where by virtue of increased charge density, counter-ions are attracted and occupy positions in the 'stern layer'. A fraction of the counter-ions is thus bound²⁶ in the region which may considerably reduce the surface charge density. Information about the counter-ion binding is necessary for the understanding of the electrical double layer as well as accurate calculation of thermodynamics of micellization to be discussed subsequently. The electrochemical methods, viz. conductance and potentiometric are employed for the estimation of counter-ion binding²⁷. The extent of binding may be as high as 90%, quite a sizeable percentage of counter-ions may bind with the micelle greatly reducing the surface charge density and hence the double layer potential. The aggregation number and other properties, environmental variations can affect the counter-ion condensation of ionic micelles.

The polarity of the interior of micelles ought to be low like hydrocarbon oils. Fluorescence measurements²⁸ with suitable probes can estimate the magnitude of the polarity. A probe sitting at the interface should send the indication about the polarity of the interface. For information about the core, the probe should be well within the interior; an oil soluble probe would serve the purpose. The physicochemical properties of micelles are presented in Table 2.

Energetics of micellization

All physicochemical processes are energetically controlled. The spontaneous formation of micelle is obviously guided by thermodynamic principle. The matter is treated under two formalisms²⁹⁻³¹: (i) mass action principle, and (ii) 'pseudophase principle'. According to the first principle, above CMC, the concentrations of monomer and micelle are interdependent. Increase of monomer concentration increases micellar concentration and vice-versa in accordance with the following equilibrium



where S^- or S^+ or S = surfactant monomer; S_n^- or S_n^+ or S_n = micelle; n = aggregation number, and K_M = micellization constant with free energy of micellization (ΔG_M^0) = $RT \ln K_M$. At CMC, by conceptual approximation, the free energy of micellization expressed per mole of monomer unit ($\Delta G_m^0 = \Delta G_M^0/n$) is given by the relation,

Table 2. Physicochemical properties of some typical surfactants

Surfactant ^a	CMC/m mol dm ⁻³	Aggregation number	% Counterion bound
SDeS ⁴⁰	41.0	30	40
SDS ²⁵	8.0	50	60
AOT ²⁵	3.0	15	10
NaC ²⁰	12.0	2	—
NaDC ²⁰	5.0	4	—
NaTC ²⁰	10.0	4	—
DTAB ³⁰	16.0	48	77
TTAB ³⁰	3.1	55	73
CTAB ³⁰	0.8	55	85
CPC ³⁰	0.83	—	58
Triton X-100 ²⁵	0.03	134	—
Tween 20 ²⁵	0.05	86	—
Tween 40 ²⁵	0.023	92	—
Tween 60 ²⁵	0.021	112	—
Tween 80 ²⁵	0.01	124	—

^aSuperscripts refer to the temperature in °C. Abbreviations as in Table 1.

$$\Delta G_m^0 = RT \ln \text{CMC}. \quad (2)$$

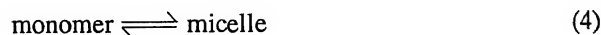
Considering counter-ion binding to ionic micelles, eqn (2) is modified to

$$\Delta G_m^0 = (1 + f) RT \ln \text{CMC}, \quad (3)$$

where f = fraction of counter-ion bound to a micelle.

For nonionic surfactants, $f = 0$ and eqn (3) is reduced to eqn (2). It is seen that in case of ionic micelles, for accurate calculation of ΔG_m^0 , determination of f is a must.

In the pseudophase model, the monomer concentration at and above CMC remains nonvariant; with increasing surfactant concentration above CMC micelles are only formed. This is like solubility of a sparingly soluble salt where above the solubility limit excess amount separates out as the insoluble phase. The micellar pseudophase on the other hand remains in solution. Based on the phase equilibrium,



at a constant temperature, chemical potential of surfactant monomer in solution (μ_m) is equal to the chemical potential of the monomer in the pseudomicellar phase (μ_M) thus,

$$\mu_m = \mu_M. \quad (5)$$

Explicitly,

$$\mu_m^0 + RT \ln a_m = \mu_M^0 + RT \ln a_M. \quad (6)$$

Wherefrom we again get

$$\Delta G_m^o = RT \ln \text{CMC} \quad (7)$$

for nonionic micelle, and

$$\Delta G_m^o = (1+f) RT \ln \text{CMC} \quad (8)$$

for ionic micelle. The μ_m^o and μ_M^o are the standard chemical potentials of monomer and micelle respectively and a_m and a_M are their corresponding activities ($a_M=1$, for pseudophase is taken to be pure phase). The two models thus lead to the same interpretation of results. It is to be noted that in the above thermodynamic treatments, at the critical micelle concentration, the equilibrium concentration of free monomer is considered equivalent to CMC.

Applying Gibbs-Helmholtz equation, the standard enthalpy of micellization (ΔH_m^o) at constant pressure, P for nonionic micelles is given by the relation

$$\Delta H_m^o = -RT^2 \left(\frac{\partial \ln \text{CMC}}{\partial T} \right)_P = R \left(\frac{\partial \ln \text{CMC}}{\partial (1/T)} \right)_P \quad (9)$$

The entropy of micellization (ΔS_m^o) follows from the Gibbs equation,

$$\Delta S_m^o = \left(\frac{\Delta H_m^o - \Delta G_m^o}{T} \right) \quad (10)$$

The factor $(1+f)$ has to be included in the above relations for ionic micelles. The measurement of CMC at different temperatures is required for the evaluation of ΔH_m^o and ΔS_m^o . It is assumed that the aggregation number and counter-ion binding of the micelle are not affected by temperature variation at least in the range of measurements, which is not large in practice. Calorimetric measurements may offer direct determination^{32,33} of both CMC (i.e. ΔG_m^o) and ΔH_m^o at a constant temperature from which ΔS_m^o readily follows.

The ΔS_m^o values are essentially positive, negative values are seldom obtained^{16,34}. The micellization process therefore ends up with increase of entropy, an overall disorder state is envisaged. This is due to the release of solvent molecules attached with the nonpolar tails of surfactant monomers by hydrophobic hydration during self organization, the entropy gain by the process exceeds the loss by amphiphile association, solvation, etc., making the overall entropy change positive.

Mixed micelles

Mixed surfactants after a critical concentration should also micellize^{5,35,36} yielding CMC. The tendency is guided

by their attractive (synergistic) and repulsive (antagonistic) interactions. This is reflected in their CMC values compared to the CMC of their components. The occurrence of mixed surfactants and hence mixed micelles are common in industrial, pharmaceutical and biological fields; physicochemically, they work better than pure surfactants in solution. A schematic representation of mixed micelles is presented in Figure 6.

It is interesting to observe that although cationic and anionic surfactants may form insoluble ion-pairs at nearly equal molar proportions, they get solubilized if the proportion of one of them is appreciably larger than the other³⁷. Thus mixed micelles of ionic-ionic, ionic-nonionic and nonionic-nonionic combinations are possible whose physicochemical studies are required for formulations, uses and basic understanding.

The CMC of ideal mixtures of surfactants is given by Client's^{5,35} equation,

$$\frac{1}{(\text{CMC})_{\text{mix}}} = \sum_i \frac{\alpha_i}{(\text{CMC})_i} \quad (11)$$

where α_i and $(\text{CMC})_i$ are the mole fraction and CMC of the i th component respectively.

In most occasions the equation is not found to be obeyed, it may take the form

$$\frac{1}{(\text{CMC})_{\text{mix}}} = \frac{\alpha_i}{f_i (\text{CMC})_i} \quad (12)$$

where the new term f_i is the activity coefficient of the i th species.

There are recent theories³⁸⁻⁴⁰ to account for the mole fraction, activity coefficient, extent of interaction among the surfactants in the mixed micelles formed. The theories need improvement to account for all kinds of combinations, but the approaches are of considerable merit⁴¹. The propositions and equations are essentially on binary combinations, for higher combinations modifications are necessary. In mixed state, fundamental studies on binary systems are considerable, ternary combinations have been rarely investigated.

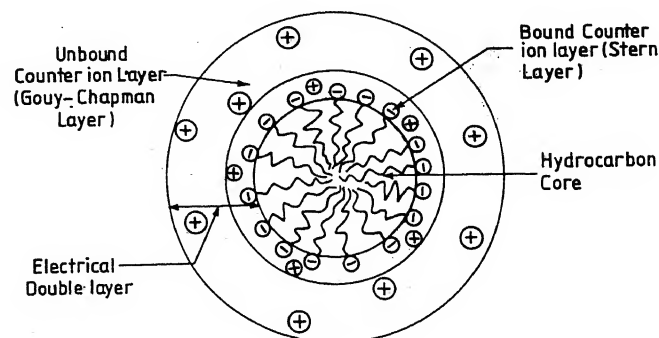


Figure 6. Schematic representation of ionic micelle showing counter-ion binding and the electrical double layer.

Reverse micelles

The micelles so far discussed are formed in aqueous medium. The surfactants may also self-assemble in non-polar solvents, viz. hydrocarbons, carbon tetrachloride, benzene, cyclohexane, chloroform, etc. They organize in a reverse manner, with their heads oriented towards the interior or core and the nonpolar tails towards the solvent. They are called 'reverse micelle'. In most occasions, the presence of water helps reverse micelles formation. Thus, there exists a tiny water core surrounded by the surfactant tails embedded in oil medium (Figure 7). Spectrophotometric, fluorimetric and calorimetric methods are used for the understanding of the beginning of their formation (i.e. CMC) and characterization⁴²⁻⁴⁴.

Dimensionally reverse micelles are comparable with normal micelles; both are thermodynamically stable. Their aggregation number and thermodynamics of formation can be assessed following the same arguments as for normal micelles. The counter-ion binding to the ionic surfactant head in the water pool of reverse micelles is difficult to estimate.

The reverse micelles can consume water or polar solvent in the interior, the core increases in size and may exceed micellar dimension. Such stable dispersions are called water-in-oil (W/O) microemulsions⁴⁵. In an analogous manner, normal micelles can accommodate oil to bulge in size forming oil-in-water (O/W) microemulsions. More addition of water and oil in the two categories of microdispersions ends up in the formation of bigger particle size yielding emulsions. Further discussion on this topic is beyond the scope of this article.

Uses of micelles

Micellar solutions are called 'compartmentalized liquids', the micelles serving as compartments which can help dissolution of polar and nonpolar compounds in normal and reverse micellar solutions respectively. This is an advantage which is not achieved otherwise. Solubilization and dispersion⁴⁶ are two important uses of micellar solution. During detergency, the micelles supply amphiphiles to act at the dirt-fabric interface for dislodging the dirt^{5,16,34} which finally is dispersed in the aqueous medium by their incorporation in the micelle for easy removal by washing.

About 50% of underground oil is recovered by pumping, and water as well as steam flooding, the rest 50% remains trapped in the pores and cracks of underground rocks which is difficult to recover. It has been established through research that by flooding micellar and microemulsion solution in the underground rocks, the interfacial tension between oil and aqueous solution can be greatly reduced to decrease the Laplace pressure under the curved oil meniscus in the pores to help mobilize

the oil for easy recovery^{16,47}. By this tertiary recovery or 'enhanced recovery' process another 30% of the underground oil can be recovered. But because of high price of surfactants etc. the method may not be on the whole cost effective. Further research in this direction has potential prospect.

The micellar solutions can influence reaction kinetics, both enhancement and retardation of the reaction rate may occur^{4,8,49}. Substrate binding and solubilization in the micelle are the main reasons for influencing the reaction kinetics. The activation barrier may be potentially affected. The reverse micelles may show significant influence on the reaction rates⁵⁰. Thus quick formation of products and often formation of new products can be achieved. Micellar kinetics are thus also compared with enzyme kinetics. It has been found that enzyme activities may also be enhanced and reduced by trapping them inside the aqueous pool of reverse micelles; the enzyme may show increased stability in compartmentalized liquids⁵¹⁻⁵³. The kinetics of substrate splitting etc. have been found to occur with altered efficiency; supra activities have also been reported. This has generated great impact of application of micellar medium-induced chemical and biochemical reaction processes.

The preparation of ultrafine monodisperse particles of colloidal dimensions is of great need in heterogeneous catalysis, magnetic tape production, biomedical application, etc. These can be conveniently prepared in compartmentalized (reverse micellar) media; thus microfine particles (often called nanoparticles) of desired sizes can be obtained through necessary chemical reactions^{54,55}. The oxides of iron, aluminium, chromium, nickel, etc.; sulphates of calcium, barium etc.; oxalates and phosphates of calcium, etc.; are examples of such materials which have prospects of preparation at the microfine monodisperse level by the above surface chemical method. The process of emulsion polymerization can be performed in reverse micelles for the preparation of polymers of controlled molecular weight and size. This is a significant advancement in the application of the micellar medium in technology.

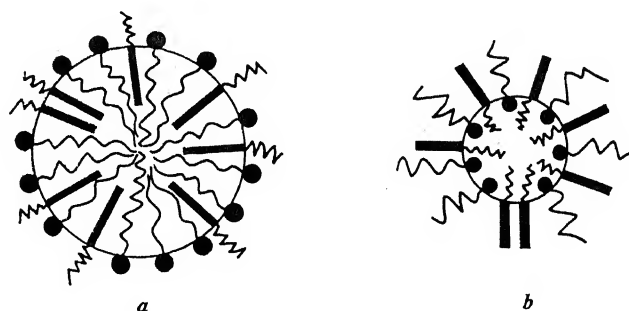


Figure 7 *a, b*. Schematic representation of mixed micelles. *a*, normal micelle; *b*, reverse micelle.

Photochemical reactions are influenced by micellar medium^{15,56}; the interfacial region can affect the photochemical pathways changing the quantum yield. For example, in photo-decarbonylation of asymmetrical diaryl ketones of general formula ACOB in micellar medium, the yield of AB is very high compared to AA and BB which is much low without micelles⁵⁷. The molecule ACOB in a micelle essentially forms AB; the probability of reaction between two such molecules in two different micelles to form AA and BB is obviously very low.

Micellar solutions have prospects in carrier-mediated transport. A material otherwise insoluble can be captured by the micelle in one solution and is transferred into another. This principle is often used in phase transfer catalysis using micelles as carriers. Separation of proteins and other polar compounds by the use of reverse micelles has been demonstrated^{58,59}.

In presence of salt, anionic micelles undergo sphere to rod transition, making the solution viscous or thick. They undergo shear thinning assisting easier application. This principle is used in the preparation of shampoo which would be otherwise too watery to apply on hair¹⁵. Control of viscosity of preparations with nonionic surfactants is done by varying the length of the hydrophilic polyoxyethylene head group for effective surface wetting and detergency.

Pollutants can be solubilized by the micelles; both organic and inorganic pollutants are amenable to such conditions. Using micelles of desired size, they can be retained by the membrane while the solution is ultrafiltered for their removal^{60,61}. The surfactants can form chelate with metal ions aided by hydrophobic association, counter-ion binding can hold organic ions on them. They can be removed as well by ultrafiltration.

Micellar medium may be utilized for stereospecific reactions; one enantiomer may be favoured over another by virtue of strong orientational effects of polar amphiphilic reactants⁶².

Despite citing above a good number of avenues for profitable utilization of micellar medium, their large scale uses have become only limited. This is because of low substrate solubility, temperature and salt instability and difficulties in the separation of products from the surfactants. It is hoped that these hurdles will be overcome through exhaustive basic research and planned trials employing newly developed surfactants; until then the field remains challengingly open to scientists and technologists. The versatility of micellar solution is the key factor for its continued study and appropriate documentation.

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Immunodiagnostic approaches to detect bovine tuberculosis

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Even though tuberculosis is an ancient disease, its early diagnosis is still a global problem. Conventional diagnostic tests along with some improved techniques and molecular biological approaches have been reviewed. The current status and the pitfalls of the diagnostic procedures are reported. An attempt has also been made to give some insight of the future prospect of the field of research.

A recent report of the World Health Organization shows that multidrug resistant tuberculosis, which often leads to death, has emerged as a new global challenge. The

WHO, which has declared TB as a global emergency, has warned that the TB crisis will continue to grow unless immediate action is taken to stop its growth. The public health risk is more in agrarian countries like India, as bovine tuberculosis is a serious problem even now. The relationship between bovine tuberculosis and human tuberculosis has been reviewed earlier and it has been established that tuberculosis in cattle may be transmitted to man via milk, milk products, meat and directly in the cowbyre¹. The resurgence of the devastating disease has led to renewed interest in the development of improved diagnostic tests for tuberculosis in animals and a review of control measures.

The basis of the TB eradication programme in other countries has been systemic application of the standard tuberculin test and the slaughter of reactors. But in India test and slaughter method cannot be used due to high incidence of reactors and moreover, this procedure would result in the loss of highly productive cows and working animals. Hence, the accurate diagnosis of the infected animals remains a major concern for eradication of the bovine tuberculosis.

Early approaches

Efforts have been made to develop *in vitro* tests for tuberculosis. The conventional methods for detection and diagnosing bovine tuberculosis have been tried. A variety of serological tests, viz. complement fixation test^{2,3}, bentonite flocculation test^{4,5}, kaolin agglutination test⁶ and indirect fluorescent antibody test⁷ have been evaluated for the diagnosis of bovine tuberculosis. But these assays are less sensitive and less specific due to antigenic cross reactivity between mycobacteria often encountered by all mammalian species.

Cellular vs humoral immune response

It is well established that tuberculosis in both human and cattle induces a strong cellular response⁸⁻¹⁰ and hence a standard test for detection of cell-mediated immune response against tuberculosis in cattle should be targeted. The reaction mediated in a tuberculous cattle as a result of an intradermal injection of tuberculin, an antigenic extract derived from the tubercle bacillus, is of delayed hypersensitivity type. The reaction to tuberculin in a sensitized animal is an immunologically specific inflammatory reaction mediated by T cells. Because positive tuberculin reaction occurs only animals that have or have had tuberculosis, skin testing may be used to identify animals infected by this disease. But the tuberculin tests fails to detect some infected humans and cattle in advanced stages of the disease and it has been reported that the antibody responses in such cases of advanced disease were elevated^{10,11}. Therefore, a serological test coupled with a cellular test should give the greatest degree of accurate diagnosis¹². Moreover, the intradermal tuberculin test is known to lack both specificity and sensitivity due, in part, to the complex nature of the antigen purified protein derivative (PPD) used, which includes several cross-reactive proteins common to a range of other mycobacteria.

Improved diagnostic tests

Several workers recommended the use of enzyme linked immunosorbent assay (ELISA) as a complementary test

with tuberculin testing. The use of ELISA for bovine tuberculosis using tuberculin PPD as the source of antigen was first reported¹³ in 1983. Subsequently, it has been reported that ELISA can be used for the detection of *Mycobacterium bovis* infected animals, anergic to the tuberculin test. The specificity of ELISAs using sonicate preparations of *M. bovis* was reported to be low¹⁴. This may be due to the presence of higher concentration of the widely cross-reactive heat-shock proteins in these preparations compared with culture filtrate preparation¹⁵.

DNA synthesis is the most commonly measured parameter used for quantitative lymphocyte blastogenesis. This is usually accomplished by measuring the uptake and incorporation of radioactivity labelled base analogue or other molecules associated with cellular DNA synthesis. ³H-thymidine uptake measures the rate of DNA synthesis. ³H-thymidine uptake by tuberculin-stimulated lymphocytes is highly correlated with the degree of delayed skin reactivity. *In vitro* lymphocyte stimulation test tend itself as a practical and reliable test to aid in the diagnosis of *M. bovis* infection in cattle¹⁶. Lymphocyte transformation test is more sensitive and often positive when the skin test is negative, doubtful or feeble¹⁷. However, the incubation time and number of laboratory manipulations make it unsuitable for field work.

Therefore, a rapid, whole blood incubation system was developed and the release of a cytokine, interferon gamma (IFN- γ), as the indicator of a positive response to *M. bovis* antigen (bovine PPD) was proposed. A bioassay to detect the presence of IFN- γ , which showed good correlation with results obtained with the lymphocyte proliferation assay was developed initially¹⁸. Subsequently, monoclonal antibodies to recombinant bovine IFN- γ were produced¹⁹. These monoclonal antibodies were used to develop a sensitive enzyme immunoassay (EIA) for bovine IFN- γ (ref. 20). The bovine IFN- γ EIA when used in conjunction with the whole blood culture system resulted in a simple, rapid (24 h) and sensitive *in vitro* assay for detecting specific cell-mediated immune responsiveness in *M. bovis*-infected cattle. The IFN- γ assay has been accredited by the Standing Committee on Agriculture as a diagnostic test for bovine tuberculosis in Australia. The IFN- γ EIA is produced commercially and field trials of this assay, for the diagnosis of bovine tuberculosis, are currently in progress in the USA, New Zealand and Ireland²¹.

Molecular biological approaches

In the last couple of years, molecular biology has provided new approaches which have enabled detailed studies to be made of the molecular characteristics of *M. bovis*. These characteristics have been investigated for their potential use in diagnosis and epidemiological

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studies. Restriction fragment analysis of genomic DNA from isolates of *M. bovis* has provided a highly discriminating typing system which has been extensively used for epidemiological studies. DNA elements in *M. bovis* have been investigated for their potential use in diagnostic assay based on the polymerase chain reaction (PCR). PCR-based diagnostic tests have the potential to detect DNA from a single organism of a predetermined species in a few days or less. This ability to provide a test that is specific and sensitive has encouraged development of many such tests for *M. tuberculosis* and these tests would also be expected to detect *M. bovis*. But unfortunately, techniques from sputum are not adequate for extracting very small number of *M. bovis* organisms from tissue lesions. Until this problem is overcome, diagnostic tests for *M. bovis* based on PCR will not have the sensitivity of current culture methods²².

Conclusion

Limited specificity and sensitivity is still a major problem in the development of a suitable diagnostic test for tuberculosis. Mycobacterial antigens consisting of a few or no crossreacting components, are needed to differentiate between tuberculous and non-tuberculous infections. In the past, efforts have been made to isolate specific antigen present in *M. bovis* BCG and *M. bovis* AN5 culture filtrates^{15,23-25}. Unfortunately, the cross reactivity of all the antigen purified so far was unacceptably high¹².

Recombinant mycobacterial antigen produced in *Escherichia coli* appears to be of limited use as diagnostic agents, because they are not consistently recognized by infected animals. The potential benefits of PCR assay approaches will remain the subject of intensive investigation. Hence, to identify the critical antigen(s) for the diagnosis of bovine tuberculosis, a lot more work is needed to improve the specificity without undue loss of sensitivity.

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Assessment of plant biodiversity at a mid-elevation evergreen forest of Kalakad–Mundanthurai Tiger Reserve, Western Ghats, India

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Plant biodiversity of an undisturbed mid-elevation evergreen forest in the southern Western Ghats was assessed by establishing 5 transects totalling 3.82 ha. All plants above 10 cm DBH were enumerated and 10% of the transect area was sampled to quantify the diversity of understorey community and to assess the regeneration status of tree species.

A total of 173 woody plant species from 58 families were recorded; of these 50% were tree species. Species diversity (H') was 4.87, ranking highest among other similar sites in the Western Ghats. On the basis of dominance, this forest is identified as *Cullenia Aglaia Palaquium* type which is considered as a subtype of *Cullenia Mesua Palaquium* series. Stem density and basal area was 363.67 (0.5 ha) and 42.03 m²/ha respectively. The 'L' shaped curve of different DBH classes indicates good regeneration in the climax forest. Small scale altitudinal changes on species composition are largely due to transition in vegetation types influenced by bioclimatic and edaphic factors.

A major impediment in documenting forest vegetation in the country has been the lack of any quantitative information. Forest vegetation has been largely described on the basis of qualitative criteria such as the physiognomy and the dominant species^{1,2}. Quantitative descriptions of vegetation, documenting the relative abundance and distribution of plant species are uncommon. Within the Western Ghats, as elsewhere in the world, species richness is high in the wet evergreen forests. Yet except for Pascal's studies, based on 0.16 to 0.20 ha plots, there is no quantitative assessment of plant biodiversity for an area that covers 28,000 km².

Apart from deforestation, forest degradation is a major problem in Indian forests. Forest Survey of India's latest report shows that 40% of the country's forest has less than 10%–40% of canopy cover³. Regeneration in many of India's forests, including the forest of Western Ghats, is inadequate to replace the adults^{4,5}. Thus in

addition to quantitative assessment of plant biodiversity, we also need to assess the regeneration status of the forest communities.

The present study is based on the mid-elevation evergreen forests in the Agasthyamalai range of the southern Western Ghats. This range has been recognized as one of the five centres of high plant diversity in India by the International Union for Conservation of Nature and Natural Resources⁶ and is well known for its species richness and endemism. The area harbours not less than 2000 plant species out of 3500 species found in Western Ghats⁷.

Previous floristic studies in Kalakad hills have been restricted to botanical explorations^{8–13} until Parthasarathy *et al.*¹⁴ undertook a quantitative study in the Sengaltheri forests in Kalakad–Mundanthurai Tiger Reserve (KMTR). However, this study was done at a lower elevation and in a transition zone between evergreen and semi-evergreen forests, and the wet evergreen forest at a higher elevation was not sampled intensively. Moreover, only large trees were sampled in 1 ha square plots. The present study makes a quantitative assessment of the evergreen forest at a higher elevation in the reserve, and it also includes all plants < 10 cm DBH.

This study is part of an overall project that seeks to assess and monitor biodiversity in the KMTR with the following objectives:

- (i) to assess and describe plant species richness of the wet evergreen forest of Kalakad.
- (ii) to describe the level of spatial heterogeneity in the composition of the forest and
- (iii) to determine if there is adequate regeneration of major tree species.

Study area

KMTR is situated in the Agasthyamalai range of the southern Western Ghats, India, approximately at

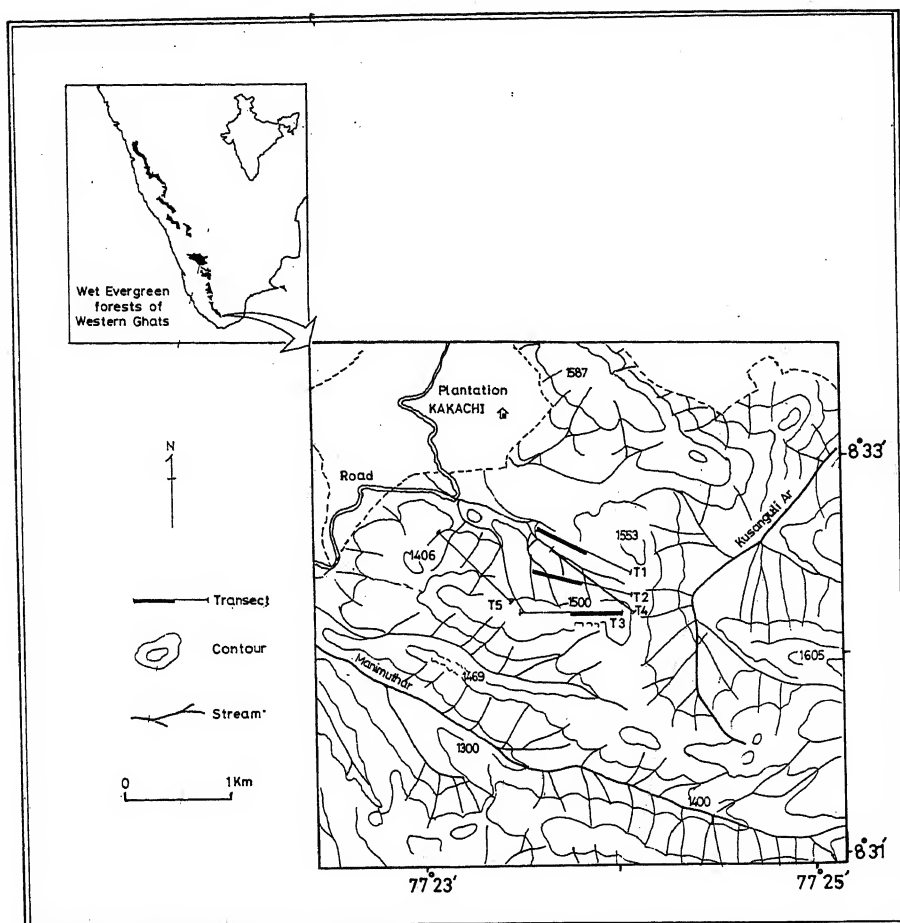


Figure 1. Map of the study area showing location of transects.

77°30'E and 8°40'N (Figure 1). The elevation of the reserve ranges from 100 to 1880 m (Agasthiar peak). The elevation gradient generates a range of vegetation from dry scrub at 150 m to dense evergreen forest above 1000 m. The study site is located near Kakachi (1250 m) in the core area of KMTR on a stretch of gently-undulating terrain, which abruptly increases in gradient forming a ridge facing northeast. The elevation of the ridge ranges from 1200 m at its base to 1550 m at its crest and is covered by dense primary forest interspersed with rock faces and *Ochlandra* facies. These forests are generally classified as belonging to the *Cullenia Mesua Palaquium* series¹⁵. The ridge occupies an area of more than 100 ha and is part of a continuous forest extending over 887 sq km of the KMTR.

The site receives over 3000 mm of rainfall from the SW and NE monsoons and six months of the year receives over 200 mm of rainfall per month (Figure 2). Temperature does not vary much over the seasons. Mean maximum temperature is 24°C and minimum about 16°C. The soil is poor in nutrients (R. Ganesan and N. Parthasarathy, unpublished) and much of the ground

is rocky. Rocky areas occupy a substantial portion of the evergreen forest.

Methods

Vegetation sampling was done along the ridge from May 1992 to September 1993. Transect method was used to sample the vegetation. Five separate transects were laid in the study area (see Figure 1). Ten plots of 10 m × 10 m were established along three 1 km transects at intervals of 100 m resulting in a 0.1 ha sample for each transect. These were laid at 1220 m (T_1), 1300 (T_2) and 1450 m (T_3) altitude respectively, parallel to the ridge. The transects could not be run strictly along the same altitudinal belt because of topographic constraints. Transect 1 (T_1) ranged from 1220 m to 1300 m, T_2 from 1300 to 1400 and T_3 from 1450 to 1550 m. A preliminary analysis of species-area relationship showed that ten 10 m × 10 m plots were not sufficient as species continued to increase linearly with area. Hence it was decided to treat the first 500 m in the above three transects as a belt of 500 m × 10 m.

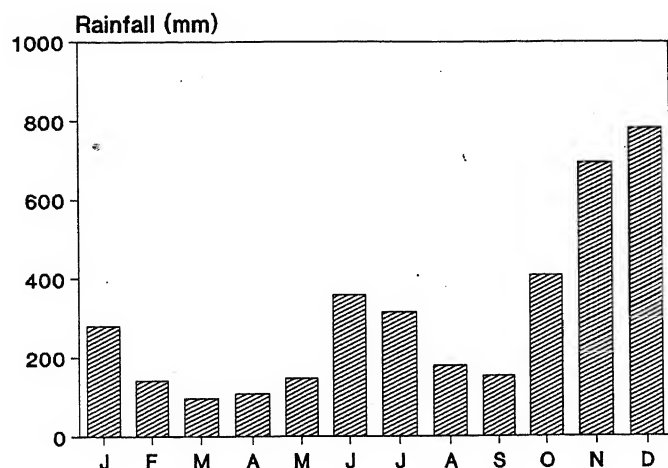


Figure 2. Mean monthly rainfall recorded at the site.

Two vertical transects of 990 m (T_4) and 940 m (T_5) long were laid descending from 1550 m to 1220 m and roughly bisecting the ridge. They are approximately parallel and 700 m away from each other. These transects could not be of 1 km length as they reached the crest at 990 m and 940 m respectively.

To sample small scale vegetation changes along altitudinal gradients, six plots were laid at intervals of 50 m elevation along the transects T_4 and T_5 . Each of these six plots had 10 subplots of 10 m \times 10 m forming a belt of 10 m \times 100 m plot. These plots were established at approximately 1200 m, 1250 m, 1300 m, 1350 m, 1400 m and 1450 m, resulting in a 0.6 ha sample for each transect. The total area sampled in this study was 3.82 ha.

All woody vegetation above 10 cm DBH at 1.3 m height was enumerated and tagged in 500 \times 10 m² belts in T_1 , T_2 and T_3 . Similarly the entire length of 990 m in T_4 and 940 m in T_5 were enumerated. All woody plants above 1 cm DBH and below 10 cm DBH were enumerated only in the 10 m \times 10 m plots at intervals of 100 m in the T_1 , T_2 and T_3 . Six such plots at intervals of 50 m altitude along T_4 and T_5 were similarly enumerated. Herbs were sampled by randomly laying 1 m² plots in the 10 m \times 10 m plots. Epiphytes were not sampled in this study. Trees and shrubs were included if their centre fell within the plot. Similarly lianas whose base fell inside the plot were enumerated. On uneven terrain, DBH was measured on the side facing the slope. Height was measured using a 2 m scale for shrubs and a pocket clinometer for trees wherever possible and visually estimated elsewhere. Altitude was recorded by a pocket altimeter with a sensitivity of 20 m.

To analyse the vegetation characteristics, two important value indices were calculated, one for species and another for family.

Species Importance Value (SIV) was calculated as follows. $SIV = \text{relative frequency} + \text{relative density} + \text{relative dominance}$.

Relative frequency = (number of plots containing a species \times 100)/sum of frequencies of all species.

Relative density = (number of individuals of a species \times 100)/total number of individuals of all species.

Relative dominance = (basal area of a species \times 100)/total basal area of all species.

The Family Importance Value (FIV) was calculated as mentioned by Keel *et al.*¹⁶. The FIV is given by

$FIV = \text{relative density} + \text{relative diversity} + \text{relative dominance}$.

Relative density = (number of individual of the species \times 100)/total number of individuals in the sample.

Relative diversity = (number of species in the family \times 100)/total number of species in the sample.

Relative dominance = (basal area of the family \times 100)/total basal area in the sample.

For lianas, shrubs and herbs, SIV was calculated by adding the relative density and relative frequency only. Specimens were collected for all species. These samples were identified in the field with the help of Gamble¹⁷ and later counter-checked with the reference material available at MH, Botanical Survey of India, Coimbatore. A reference collection of specimens with flowers/fruits was made and preserved as herbarium material.

Results

Floristics and forest structure

A total of one hundred and seventy three species of plants were recorded from the five transects representing 136 genera and 58 families. Canopy and understorey trees accounted for 90 species in 35 families, shrubs (height < 5m, DBH > 1 cm) 50 species in 17 families, herbs (excluding grasses) 18 species in 14 families and lianas 15 species in 11 families. When the species abundance was represented in octaves and the number of species on an arithmetic scale, the resulting pattern was seen to follow the standard log normal distribution (Figure 3). The best fit curve $S_r = 17e^{-(0.246r)^2}$ followed the observed distribution closely towards the higher end of the scale (chi square = 2.41 $p < 0.01$). At the lower end there were still 7 species which are rare and do not occur in the present sample. Maximum diversity was seen among canopy and understorey trees (Shannon diversity index 4.87).

Trees

Lauraceae, Rubiaceae and Euphorbiaceae were the three most dominant families in terms of species richness in the forest (Figure 4). However FIV index for pooled

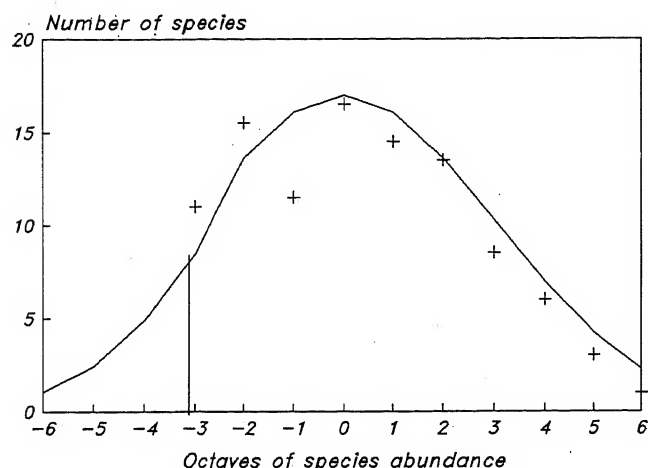


Figure 3. Log normal distribution of species abundance (> 10 cm DBH).

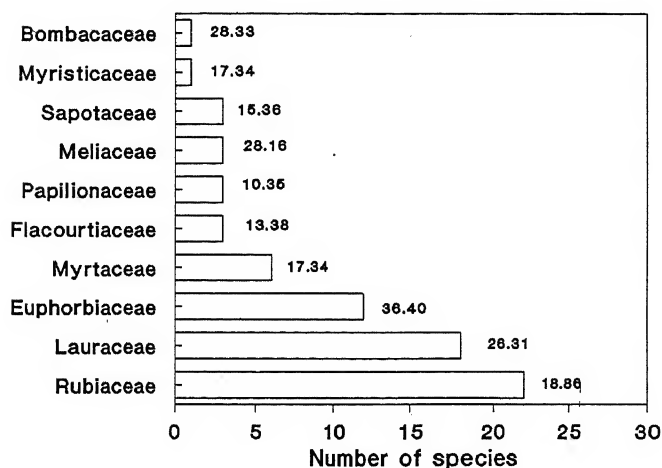


Figure 4. Family dominance based on species richness. FIV values are given at the end of each bar.

data from all five transects showed that Euphorbiaceae was the most important family with an FIV value of 36.4 followed by Bombacaceae (28.33) and Lauraceae (26.31) (Figure 4). Euphorbiaceae dominated the understorey and only *Macaranga peltata* and *Mallotus tetracoccus* reached the canopy and subcanopy.

Bombacaceae was represented by only one species – *Cullenia exarillata* but because of its larger DBH and high density, it was the second dominant family. In contrast, Lauraceae and Rubiaceae in spite of their high species richness, do not have high FIV value because of their lower density and lower basal areas. Thirty one families were represented by just one species and only sixteen families had more than 3 species. Lauraceae was dominated by trees (16 out of 18 species) and all of them occur at very low abundance. Among Rubiaceae, except for *Tricalysia apiocarpa* and *Canthium ficiforme*,

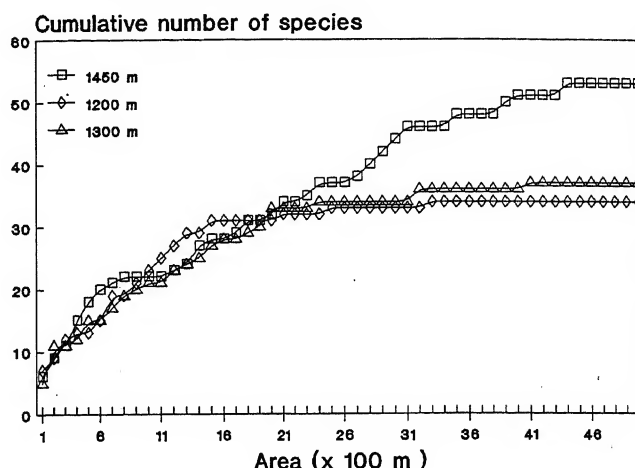


Figure 5. Species-area relationship of trees > 10 cm DBH.

all other members were shrubs with less than 10 cm DBH.

The species area curve constructed to determine the adequacy of sampling in the three transects showed that very few species are added after 45 plots (Figure 5). For transect T_1 and T_2 such saturation occurred in less than 40 plots. In T_3 more number of plots were needed for the same. As each transect sampled 5000 m² (0.5 ha) of forest (of fifty 100 m², contiguous plots), it appears that by 4500 m², most of the species found in the patch are encountered.

The 0.5 ha sample from T_1 , T_2 and T_3 was used for comparison between transects. Species diversity was higher in T_3 than T_1 (Table 1). Morisita Horn index (measure of beta diversity) showed 19% similarity between the T_3 and T_1 (Morisita Horn index = 0.1893) but 50% similarity between T_1 and T_2 (Morisita Horn index = 0.5004).

From the SIV values calculated with pooled data, *Cullenia exarillata*, *Aglaia elaeagnoidea* var. *bourdillonii* and *Palaquium ellipticum* emerged as the most important species in the forest (Table 2). *A. elaeagnoidea* had the highest density among them. SIV values are influenced by relative dominance and relative density to various degrees for each species. For large trees like *C. exarillata* and *P. ellipticum*, relative dominance accounted for 70% of the SIV value whereas for *A. elaeagnoidea*, although being abundant, accounted for only 32%, because of its smaller girth.

Palm diversity was very low inside the forest. Only one species, *Bentinckia codapanna* with two saplings was encountered. *Nageia wallichiana* (Podocarpaceae), the only gymnosperm in the forest, had only seven individuals (> 10 cm DBH) from the 2708 stems sampled.

Stem density and basal area are shown in Table 1. The first 10 dominant species in the forest accounted for 65% (1496) of the stems sampled, 31% (744) of these

Table 1. Dominant tree species, stem density, basal area and species diversity of different life-forms in the three horizontal transects of 0.5 ha. Number of species is given in parenthesis

Transect	Dominant tree species	Stem density	Basal area (m ²)	Species diversity*			
				Tree	Shrub	Herb	Liana
1250 m	<i>Cullenia exarillata</i> <i>Palaquium ellipticum</i> <i>Agrostistachys borneensis</i>	358	30.01	3.89 (31)	3.72 (27)	1.76 (4)	–
1300 m	<i>Cullenia exarillata</i> <i>Aglaia elaeagnoidea</i> <i>Agrostistachys borneensis</i>	315	27.43	3.96 (36)	3.07 (19)	2.06 (5)	1.79 (5)
1450 m	<i>Aglaia elaeagnoidea</i> <i>Alseodaphne semicarpifolia</i> <i>Hydnocarpus alpina</i>	418	42.35	4.47 (48)	2.47 (29)	2.96 (9)	2.23 (6)

*Shannon–Wiener diversity index calculated to base 2.

are accounted by the top three species, viz *C. exarillata*, *A. elaeagnoidea* var. *bourdillonii* and *P. ellipticum*.

The DBH distribution from pooled data shows a typical 'L' shaped curve (Figure 6). Though T_1 and T_2 did not differ in their distribution (Kolmogorov–Smirnov test $D = 0.0305$ $p < 0.05$), T_3 differed significantly from T_1 ($D = 0.096$ $p < 0.05$) and T_2 ($D = 0.92$ $p < 0.05$). However the general similarity of the 'L' shaped curve in all the transects indicates the undisturbed nature of the forest stand.

The change in abundance of the dominant species along the elevational gradient at intervals of 50 m is shown in Figures 7a and b. Based on their abundance at different elevations, they were classified into two categories; low elevation and high elevation species.

The low elevation species: *Palaquium ellipticum*, *Cullenia exarillata*, *Myristica dactyloides*, *Epiprinus malloiformis*, *Artocarpus heterophyllus*, *Holigarna nigra* and *Elaeocarpus tuberculatus* were found in both the transects but restricted to elevation < 1300 m with the exception of *E. malloiformis* in transect 5.

The high elevation species: Trees like *Pygeum sisparense*, *Alseodaphne semicarpifolia*, *Memecylon malabaricum* and *Syzygium densiflorum* were restricted to above 1400 m whereas *Hydnocarpus alpina*, *Drypetes longifolia*, *Mastixia arborea* also found elsewhere, occurred at a higher density at this altitude.

Shrubs (1 m–5 m)

Over 50 species of shrubs were encountered in the area, dominated by Rubiaceae 14 spp. (30%) and Acanthaceae 9 spp. (18%). Shrub diversity ($H' = 3.91$) was significantly less than tree diversity ($H' = 4.17$; $t = 20.29$, $df = 2725$ $p < 0.001$). Dominant species were *Nilgirianthus foliosus*, *N. perrottetianus*, *Diotacanthus grandis* and *Agrostistachys indica*. The latter two species were common in all the three transects (Table 2).

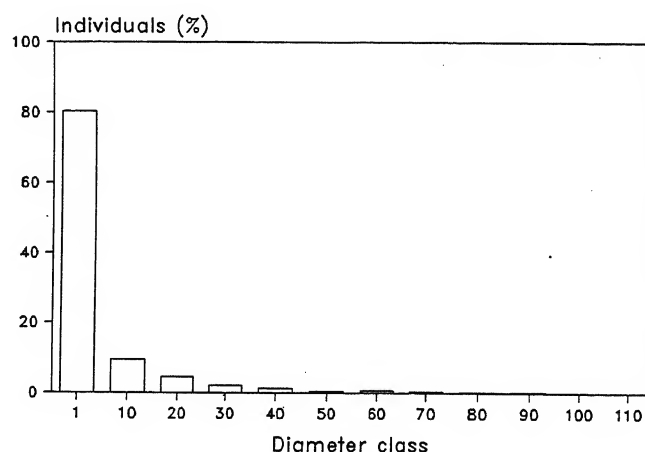


Figure 6. Size class distribution of all individuals in the transects.

Elevational changes in diversity showed a decrease from 1250 m (T_1) to 1450 m (T_3) (Table 1). Shrub density at every 50 m altitudinal interval also showed a maximum density at 1250 m.

Herbs (<1 m)

The herb community was not dominated by any single family. Families like Rubiaceae, Euphorbiaceae and Zingiberaceae had 2 to 3 species while others were monospecific. Eighteen species of herbs were recorded. Only *Curculigo orchioides* was common to all transects (Table 2).

Elevational changes in diversity increased from lower to higher elevations (Table 2), while abundance shows a high at 1250 m altitude and a low at 1300 m followed by a steady rise further up. Pteridophytes like ferns were found in greater density in the lower altitudes (T_1 and T_2).

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Table 2. Floristic composition, frequency, density and SIV of different life-forms sampled from all five transects. The variety names of the species are given as footnote.

Species	Frequency	Pooled data		SIV
		Density	Basal area (cm ²)	
Trees				
<i>Cullenia exarillata</i> Robyns. (Bombacaceae)	138	243	514977.37	39.60
<i>Aglaia elaeagnoidea</i> (Juss.) Benth. ^a (Meliaceae)	181	419	171726.27	33.30
<i>Palaquium ellipticum</i> (Dalz.) Baillon (Sapotaceae)	68	104	178361.29	15.59
<i>Hydnocarpus alpina</i> Wight (Flacourtiaceae)	82	114	77912.93	12.29
<i>Myristica dactyloides</i> Gaertn. (Myristicaceae)	75	99	93727.58	12.04
<i>Tricalysia apiocarpa</i> (Dalz.) Gamble (Rubiaceae)	56	69	128838.17	11.42
<i>Alseodaphne semicarpifolia</i> Nees ^b (Lauraceae)	30	41	151195.95	9.91
<i>Syzygium gardneri</i> Thw. (Myrtaceae)	47	62	61803.26	7.68
<i>Calophyllum austroindicum</i> Kosterm. ex Stevens (Guttiferae)	32	32	86410.73	6.82
<i>Artocarpus heterophyllus</i> Lam. (Moraceae)	51	51	42216.82	6.63
<i>Diospyros malabarica</i> (Desr.) Kostel. (Ebenaceae)	29	35	45593.96	4.95
<i>Holigarna nigra</i> Bourd. (Anacardiaceae)	36	44	28028.56	4.90
<i>Ormosia travancorica</i> Bedd. (Papilionaceae)	19	22	54047.17	4.28
<i>Mastixia arborea</i> (Wight) Bedd. (Cornaceae)	24	30	24369.06	3.54
<i>Neolitsea fischeri</i> Gamble (Lauraceae)	13	14	45937.94	3.29
<i>Cinnamomum travancoricum</i> Gamble (Lauraceae)	16	19	30006.26	2.93
<i>Cryptocarya lawsonii</i> Gamble (Lauraceae)	23	25	13968.43	2.84
<i>Casearia ovata</i> (Lam.) Willd. (Flacourtiaceae)	23	23	12493.28	2.70
<i>Scolopia crenata</i> (Wight & Arn.) Clos (Flacourtiaceae)	17	18	23549.88	2.67
<i>Elaeocarpus munronii</i> (Wight) Mast. (Elaeocarpaceae)	12	18	25154.00	2.46
<i>Memecylon malabaricum</i> (Clarke) Cogn. (Melastomataceae)	17	22	13896.50	2.39
<i>Beilschmiedia wightii</i> (Nees) (Lauraceae)	14	14	9174.14	1.71
<i>Elaeocarpus tuberculatus</i> Roxb. (Elaeocarpaceae)	9	9	13435.78	1.44
<i>Canthium ficiforme</i> Hook. f. (Rubiaceae)	9	9	13168.98	1.42
<i>Macaranga peltata</i> (Roxb.) Muell.-Arg. (Euphorbiaceae)	10	10	5827.31	1.19
<i>Vepris bilocularis</i> (Wight & Arn.) Engler (Rutacea)	6	7	11196.63	1.09
<i>Celtis timorensis</i> Spamoghe (Ulmaceae)	7	7	4800.16	0.87
<i>Rapanea wightiana</i> (Wall. ex DC) Mez. (Myrsinaceae)	8	8	2598.53	0.86
<i>Nageia wallichiana</i> (Presl.) Kuntze. (Podocarpaceae)	7	7	2613.12	0.77
<i>Persea macrantha</i> (Nees) Kosterm. (Lauraceae)	5	6	3225.74	0.65
<i>Gordonia obtusa</i> Wall. ex Wight & Arn. (Theaceae)	3	3	3934.58	0.45
<i>Trichilia connaroides</i> (Wight & Arn.) Benth. (Meliaceae)	4	4	1406.56	0.44
<i>Litsea wightiana</i> (Nees) Hook. f. (Lauraceae)	2	4	2410.94	0.37
<i>Glochidion fagifolium</i> Bedd. (Euphorbiaceae)	2	3	2687.61	0.34
<i>Litsea insignis</i> Gamble (Lauraceae)	1	1	2340.00	0.20
<i>Eugenia floccosa</i> Bedd. (Myrtaceae)	1	3	633.08	0.20
<i>Dysoxylum malabaricum</i> Bedd. ex Hiern (Meliaceae)	1	1	1655.35	0.17
<i>Michelia nilagirica</i> Zenk. (Magnoliaceae)	1	1	1098.03	0.14
<i>Ternstroemia japonica</i> (Thunb.) Thunb. (Theaceae)	1	1	1017.86	0.14
<i>Litsea glabrata</i> (Wall. ex Nees) Hook. f. (Lauraceae)	1	1	835.03	0.13
<i>Mallotus tetracoccus</i> (Roxb.) Kurz (Euphorbiaceae)	1	1	459.96	0.11
<i>Prunus ceylanica</i> (Wight) Miq. (Rosaceae)	1	1	174.28	0.10
Understorey trees				
<i>Agrostistachys borneensis</i> Becc. (Euphorbiaceae)	129	236	67852.86	19.00
<i>Gomphandra coriacea</i> Wight (Icacinaceae)	108	147	39164.89	13.26
<i>Drypetes longifolia</i> (Blume) Pax & Hoffm. (Euphorbiaceae)	69	118	42864.61	10.15
<i>Xanthophyllum flavescens</i> Roxb. (Xanthophyllaceae)	22	79	49524.49	6.35
<i>Epiprinus mallotiformis</i> (Muell.-Arg.) Croizat (Euphorbiaceae)	48	66	13638.24	5.75
<i>Antidesma menasu</i> (Tul.) Miq. ex Muell.-Arg. (Stilaginaceae)	33	42	8050.51	3.77
<i>Acronychia pedunculata</i> (L.) Miq. (Rutaceae)	29	40	8551.20	3.49
<i>Syzygium mundagam</i> (Bourd.) Chithra (Myrtaceae)	28	32	9461.44	3.18
<i>Ixora nigricans</i> R. Br. ex Wight & Arn. (Rubiaceae)	9	65	1837.83	2.99
<i>Litsea ligustrina</i> (Nees) Hook. f. (Lauraceae)	16	20	4656.67	1.85
<i>Clerodendrum viscosum</i> Vent. (Verbenaceae)	11	24	6819.07	1.81
<i>Glycosmis pentaphylla</i> (Retz.) DC. (Rutacea)	16	17	3731.35	1.70

contd....

Table 2. contd....

Species	Frequency	Pooled data		SIV
		Density	Basal area (cm ²)	
<i>Syzygium benthamianum</i> (Wight ex Duthie) Gamble (Myrtaceae)	13	16	6167.77	1.60
<i>Syzygium densiflorum</i> Wall. ex Wight & Arn. (Myrtaceae)	6	13	16063.38	1.53
<i>Nothopodia travancorica</i> Bedd. ex Hook. f. (Anacardiaceae)	12	17	2223.02	1.40
<i>Cinnamomum sulphuratum</i> Nees (Lauraceae)	11	16	2072.12	1.30
<i>Mallotus resinosa</i> (Blanco) Merr. (Euphorbiaceae)	12	13	2655.36	1.27
<i>Euphorbia antiquorum</i> L. (Euphorbiaceae)	10	10	6052.31	1.20
<i>Pygeum sisparens</i> Gamble (Rosaceae)	10	14	1122.02	1.13
<i>Gomphia serrata</i> (Gaertn.) Kanis (Ochnaceae)	9	11	1892.49	1.00
<i>Viburnum punctatum</i> Buch.-Ham. ex D. Don (Caprifoliaceae)	8	8	4457.03	0.94
<i>Miliusa wightiana</i> Hook. f. & Thomas. (Annonaceae)	5	5	10627.87	0.94
<i>Atalantia wightii</i> Tanaka (Rutaceae)	4	4	11745.09	0.89
<i>Eugenia rotteriana</i> Wight & Arn. (Myrtaceae)	6	8	4244.71	0.82
<i>Vernonia travancorica</i> Hook. f. (Compositae)	7	7	1164.41	0.70
<i>Murraya paniculata</i> (L.) Jack (Rutaceae)	4	12	334.78	0.68
<i>Actinodaphne bourdillonii</i> Gamble (Lauraceae)	5	6	2074.49	0.60
<i>Memecylon flavescens</i> Gamble (Melastomataceae)	4	4	4592.89	0.58
<i>Tarenna asiatica</i> (L.) Kuntze ex K. Schum. (Rubiaceae)	4	5	1145.19	0.46
<i>Eugenia thwaitesii</i> Duthie (Myrtaceae)	4	6	91.23	0.45
<i>Neolitsea cassia</i> (L.) Kosterm. (Lauraceae)	4	6	145.68	0.45
<i>Isonandra perrottetiana</i> A. DC. (Sapotaceae)	4	4	592.12	0.40
<i>Octotropis travancorica</i> Bedd. (Rubiaceae)	4	4	377.76	0.39
<i>Schefflera wallichiana</i> (Wight & Arn.) Harms. (Araliaceae)	3	4	1697.43	0.39
<i>Apollonias arnottii</i> Nees (Lauraceae)	3	4	438.26	0.34
<i>Eugenia maboides</i> Wight (Myrtaceae)	3	3	913.40	0.32
<i>Nothopodytes nimmoniana</i> (Graham) Mabberley (Icacinaeae)	3	3	651.94	0.31
<i>Litsea nigrescens</i> Gamble (Lauraceae)	2	2	1705.81	0.26
<i>Maesa indica</i> (Roxb.) DC. (Myrsinaceae)	2	2	461.84	0.21
<i>Ligustrum perrottetii</i> DC. (Oleaceae)	1	1	2376.79	0.20
<i>Isonandra lanceolata</i> Wight (Sapotaceae)	2	2	191.91	0.20
<i>Olea dioica</i> Roxb. (Oleaceae)	1	1	735.71	0.13
<i>Ficus virens</i> Ait. (Moraceae)	1	1	115.04	0.10
<i>Garcinia travancorica</i> Bedd. (Guttiferae)	1	1	220.98	0.10
<i>Vaccinium leschenaultii</i> Wight ^c (Vacciniaceae)	1	1	156.21	0.10
<i>Pavetta thomsonii</i> Bremek ^d (Rubiaceae)	1	1	109.40	0.10
<i>Canthium travancoricum</i> (Bedd.) Hook. f. (Rubiaceae)	1	1	95.07	0.10
<i>Goniothalamus wightii</i> Hook. f. & Thoms. (Annonaceae)	1	1	120.81	0.10
Total	1773	2708	2254894.56	300.00
Shrubs				
<i>Nilgiranthus foliosus</i> (Wight) Bremek. (Acanthaceae)	16	815		27.33
<i>Agrostistachys indica</i> Dalz. (Euphorbiaceae)	23	321		14.07
<i>Diotaeanthus grandis</i> (Bedd.) Benth. ex Clarke (Acanthaceae)	20	316		13.33
<i>Psychotria connata</i> Wall. (Rubiaceae)	36	197		12.97
<i>Nilgiranthus perrottetianus</i> (Nees) Bremek. (Acanthaceae)	12	313		11.66
<i>Lasianthus cinereus</i> Gamble (Rubiaceae)	28	201		11.50
<i>Saprosma corymbosum</i> (Bedd.) Bedd. (Rubiaceae)	22	222		10.94
<i>Cinnamomum filipedicellatum</i> Kosterm. (Lauraceae)	15	142		7.18
<i>Calamus brandisii</i> Beccari ex Beccari & Hook. f. (Arecaceae)	12	83		4.84
<i>Psychotria anamalayana</i> Bedd. (Rubiaceae)	16	48		4.59
<i>Phyllanthus fimbriatus</i> (Wight) Muell.-Arg. (Euphorbiaceae)	18	25		4.31
<i>Micrococca beddomei</i> (Hook. f.) Prain (Euphorbiaceae)	16	22		3.82
<i>Psychotria nigra</i> (Gaertn.) Alston. (Rubiaceae)	10	51		3.49
<i>Nilgiranthus punctatus</i> (Nees) Bremek. (Acanthaceae)	6	65		3.12
<i>Ochlandra scriptoria</i> (Dennst.) Fischer (Poaceae)	3	82		3.03
<i>Hedyotis purpurascens</i> Hook. f. (Rubiaceae)	9	23		2.46
<i>Xenacanthus pulneyensis</i> (Clarke) Bremek. (Acanthaceae)	5	49		2.44
<i>Ardisia pauciflora</i> Heyne ex Roxb. (Myrsinaceae)	9	19		2.35

contd....

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Table 2. contd....

Species	Frequency	Pooled data		SIV
		Density	Basal area (cm ²)	
<i>Lasianthus strigillosus</i> Hook. f. (Rubiaceae)	8	23		2.27
<i>Symplocos wyanadense</i> (Kuntze) Nootb. (Symplocaceae)	9	16		2.26
Unidentified	5	38		2.12
<i>Calamus pseudo-tenuis</i> Beccari ex Beccari & Hook.f. (Arecaceae)	7	18		1.92
<i>Euonymus crenulatus</i> Wall. ex Wight & Arn. (Celastraceae)	7	10		1.68
<i>Goldfussia tristis</i> Wight (Acanthaceae)	3	36		1.66
<i>Microtropis stocksii</i> Gamble (Celastraceae)	7	8		1.62
<i>Leptacanthus rubicundus</i> Nees (Acanthaceae)	3	32		1.54
<i>Meiogyne pannosa</i> (Dalz.) Sinclair (Annonaceae)	6	6		1.37
<i>Chasalia curviflora</i> (Wall. ex Kurz) Thw. (Rubiaceae)	6	6		1.37
Unidentified	5	9		1.26
<i>Mussaenda hirsutissima</i> (Hook.f.) Hutchinson ex Gamble (Rubiaceae)	5	5		1.14
<i>Osbeckia aspera</i> (L.) Blume (Melastomataceae)	5	5		1.14
<i>Litsea</i> sp. (Lauraceae)	5	5		1.14
<i>Barleria involucreata</i> Nees ^a (Acanthaceae)	4	8		1.03
<i>Rauvolfia densiflora</i> (Wall.) Benth. ex Hook. f. (Apocyanaceae)	4	5		0.94
<i>Solanum</i> sp. (Solanaceae)	3	3		0.68
<i>Excoecaria crenulata</i> Wight (Euphorbiaceae)	2	9		0.66
<i>Erythroxylum obtusifolium</i> (Wight) Hook. f. (Erythroxylaceae)	2	6		0.57
<i>Lasianthus</i> sp. (Rubiaceae)	2	4		0.51
<i>Croton laccifer</i> L. (Euphorbiaceae)	2	3		0.48
<i>Didyposandra lurida</i> (Wight) Bremek. (Acanthaceae)	2	2		0.46
<i>Symplocos nairii</i> Henry, Gopalan & Swamin. (Symplocaceae)	1	1		0.23
<i>Pittosporum tetraspermum</i> Wight & Arn. (Pittosporaceae)	1	1		0.23
<i>Medinilla malabarica</i> Bedd. (Melastomataceae)	1	1		0.23
<i>Byrsophyllum tetrandrum</i> (Bedd.) Hook.f. ex Bedd. (Rubiaceae)	1	1		0.23
<i>Actinodaphne campanulata</i> Hook. f. (Lauraceae)	1	1		0.23
<i>Neurocalyx calycinus</i> (R. Br. ex Benn.) Robins (Rubiaceae)	1	1		0.23
<i>Lobelia nicotianifolia</i> Roth ex Schultes (Lobeliaceae)	1	1		0.23
<i>Clausena indica</i> (Dalz.) Oliver (Rutaceae)	1	1		0.23
<i>Memecylon subcordatum</i> Cogn. (Melastomataceae)	1	1		0.23
<i>Polyscias acuminata</i> (Wight) Seem. (Araliaceae)	1	1		0.23
Herbs				
<i>Curculigo orchiioides</i> Gaertn. (Hypoxidaceae)	11	12		2.67
Grasses	8	8		1.92
<i>Rungia wightiana</i> Nees (Acanthaceae)	7	10		1.77
<i>Elastostema lineolatum</i> Wight ^f (Urticaceae)	4	6		1.02
<i>Lycianthus laevis</i> (Dunal) Bitter (Solanaceae)	3	4		0.75
<i>Dorstenia indica</i> Wall. ex Wight (Moraceae)	2	2		0.48
<i>Oldenlandia</i> sp. (Rubiaceae)	2	2		0.48
<i>Phyllanthus</i> sp. (Euphorbiaceae)	2	2		0.48
<i>Plectranthus malabaricus</i> (Benth.) Willemse (Labiatae)	2	2		0.48
<i>Elettaria cardamomum</i> L. (Zingiberaceae)	1	3		0.30
<i>Pouzolzia</i> sp. (Urticaceae)	1	2		0.27
<i>Sonerila</i> sp. (Melastomataceae)	1	2		0.27
<i>Selaginella</i> sp. (Pteridophyte)	1	2		0.27
<i>Curculigo trichocarpa</i> (Wight) Bennet & Raizada (Hypoxidaceae)	1	1		0.24
<i>Ophiorrhiza grandiflora</i> Wight (Rubiaceae)	1	1		0.24
<i>Zingiber roseum</i> (Roxb.) Roscoe (Zingiberaceae)	1	1		0.24
<i>Anaphyllum wightii</i> Schott. (Araceae)	1	1		0.24
<i>Begonia malabarica</i> Lam. (Begoniaceae)	1	1		0.24
<i>Cyanotis arachnoidea</i> Clarke (Commelinaceae)	1	1		0.24
Lianas				
<i>Toddalia asiatica</i> (L.) Lam. (Rutaceae)	8	16		2.15
<i>Zanthoxylum tetraspermum</i> Wight & Arn. (Rutaceae)	5	9		1.32

contd....

Table 2. contd....

Species	Pooled data		Basal area (cm ²)	SIV
	Frequency	Density		
<i>Embelia basaal</i> (Roem. & Schultes) A.DC. (Myrsinaceae)	4	7		1.05
<i>Connarus wightii</i> Hook. f. (Connaraceae)	3	7		0.84
<i>Jasminum azoricum</i> L. (Oleaceae)	3	3		0.72
<i>Tylophora subramanii</i> Henry (Asclepiadeaceae)	2	4		0.54
<i>Tetrastigma leucostaphylum</i> (Dennst.) Alston (Vitaceae)	2	2		0.48
<i>Elaeagnus kologa</i> Schlecht. (Elaeagnaceae)	2	2		0.48
<i>Oxyceros rugulosus</i> (Thw.) Tirvengadam (Rubiaceae)	1	1		0.24
<i>Ficus amplocarpa</i> Govindarajulu & Masilamoney (Moraceae)	1	1		0.24
<i>Piper</i> sp. (Piperaceae)	1	1		0.24
<i>Derris benthamii</i> (Thw.) Thw. (Papilionaceae)	1	1		0.24
<i>Segetia hamosa</i> Brongn. (Rhamnaceae)	1	1		0.24
<i>Jasminum malabaricum</i> Wight (Oleaceae)	1	1		0.24
<i>Canthium angustifolium</i> Roxb. (Rubiaceae)	1	1		0.24
Total	506	3374		200.00

a = var. *bourdillonii* (Gamble) K. K. N. Nair

b = var. *parvifolia* Hook. f.

c = var. *zeylanica* Clarke

d = var. *puberula* Bremek.

e = var. *elata* (Dalz.) Clarke

f = var. *linearis* Wedd.

Lianas

The forest was poorly represented by large lianas. Only 15 species of lianas were recorded, of which ten species occurred in the > 10 cm DBH class. These constituted only 1% (25 out of 2733 stems) of the forest stand and all occurred at low densities. The Shannon value was 3.05. *Embelia basaal* was the only common liana with a density of 1.83 per ha. Elevation-wise liana diversity increased from T_2 to T_3 , T_1 at 1200 m did not have any large class liana (Table 1).

Comparison of lifeforms in 1 m² subplots among the three transects showed that herbs and shrubs had greater representation in T_3 . Species richness among trees, lianas, shrubs and herbs, however, did not show any significant differences between them (chi square = 5.475, df = 6, $p < 0.05$).

Tree saplings

Most of the dominant species had good representation in the saplings except *P. ellipticum* which did not have many adults and saplings (Table 3). While 7 species did not have any saplings, 8 species did not have any adults but some of them, *Nothopegia travancorica* and *Neolitsea fischeri* had large number of saplings in the 3000 m² area.

Aglaia elaeagnoidea, *Hydnocarpus alpina* and *Gomphandra coriacea* are the common sapling species found in all the three elevations. Frequency of tree sapling

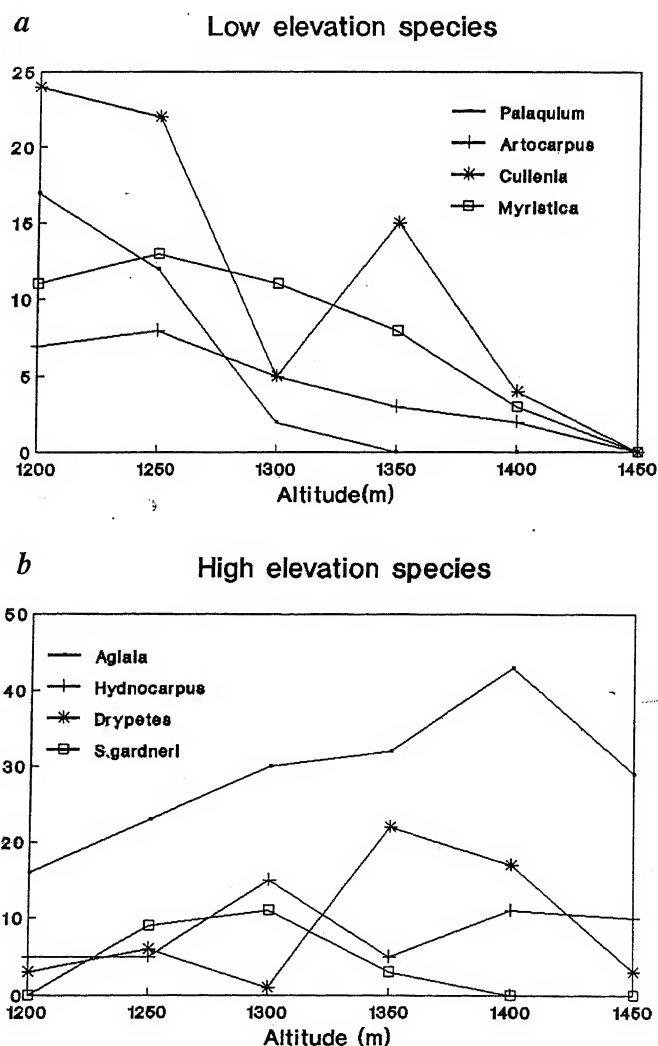


Figure 7 a, b. Distribution of dominant tree species along elevational gradients. a, Low elevation species; b, High elevation species.

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Table 3. Comparison of tree and sapling density in three horizontal transects (0.1 ha)

Species	Transect 1			Transect 2			Transect 3			Pooled		
	Adult	Sapling	SIV	Adult	Sapling	SIV	Adult	Sapling	SIV	Adults	Sapling	SIV*
<i>Cullenia exarillata</i>	12	25	38.87	7	17	61.79				19	42	40.18
<i>Aglaia elaeagnoidea</i> var. <i>bourdillonii</i>	14	44	35.98	11	55	81.2	12	3	27.59	37	102	31.83
<i>Palaquium ellipticum</i>	2	7	11.11	1		6.32				3	7	19.25
<i>Agrostistachys borneensis</i>	11	90	31.55	3	45	8.6				14	135	18.94
<i>Gomphandra coriacea</i>	2	9	5.45	2	6	8.84	3	1	10.03	7	16	14.53
<i>Myristica dactyloides</i>	7	16	19.56	1	6	3.97				8	22	14.27
<i>Hydnocarpus alpina</i>	1	1	2.86	2	10	13.31	18	10	38.79	21	21	12.40
<i>Syzygium gardneri</i>	2	11	2.86	1	8	15.84				3	19	8.48
<i>Calophyllum austroindicum</i>					8		2		10.83	2	8	7.24
<i>Artocarpus heterophyllus</i>	1	4	7.76							1	4	7.22
<i>Epiprinus mallotiformis</i>	3		9.94	2	8	21.13				5	8	6.48
<i>Holigarna nigra</i>	3	4	13.70	1		6.63				4	4	5.59
<i>Alseodaphne semecarpifolia</i>							7	3	51.79	7	3	4.72
<i>Acronychia pedunculata</i>				1	1	3.97	2	10	5.90	3	11	4.35
<i>Drypetes longifolia</i>	6	9	13.70	4	11	4.02				10	20	4.35
<i>Antidesma menasu</i>	6	11	17.13	3	8	21.93				9	19	3.88
<i>Syzygium mundagam</i>	1		3.22	1	1	5.42				2	1	3.51
<i>Tricalysia apiocarpa</i>				2	6	17.69	4	1	12.15	6	7	2.52
<i>Scolopia crenata</i>	2	1	6.45							2	1	2.49
<i>Cinnamomum travancoricum</i>	1	38	3.96					9		1	47	2.43
<i>Clerodendrum viscosum</i>	1	14	2.79							1	14	2.09
<i>Litsea ligustrina</i>							1	1	2.98	1	1	2.07
<i>Elaeocarpus tuberculatus</i>	1	6	3.39							1	6	1.72
<i>Cinnamomum filipedicellatum</i>	2	10	6.56							2	10	1.45
<i>Rapanea wightiana</i>							6	2	17.54	6	2	1.28
<i>Macaranga peltata</i>	1	3	6.73							1	3	1.24
<i>Viburnum punctatum</i>	1		3.03		1					1	1	1.02
<i>Gomphia serrata</i>	1		2.81					4		1	4	0.91
<i>Eugenia floccosa</i>							1	2	3.71	1	2	0.61
<i>Beilschmiedia wightii</i>	2	1	2.79							2	1	0.58
<i>Litsea wightiana</i>	3	9	8.74	1		4.86				4	9	0.47
<i>Glochidion fagifolium</i>				2		6.27		1		2	1	0.46
<i>Octotropis travancorica</i>				1	45	3.96				1	45	0.42
<i>Syzygium densiflorum</i>							13	4	39.49	13	4	0.23
<i>Tetrastigma lecuostaphylum</i>				1	10	4.02				1	10	0.22
<i>Fagraea ceilanica</i>							1	2	2.94	1	2	0.21
<i>Memecylon flavescens</i>							1	1	3.83	1	1	0.16
<i>Eugenia maboides</i>							1	14	3.17	1	14	0.12

* SIV values from 5 transects.

distribution showed more resemblance between lower and middle elevation (T_1 and T_2) when compared to higher elevations (T_3) (Table 3). In T_3 , saplings were dominated by *Eugenia maboides*, *Hydnocarpus alpina*, while some dominant species like *Aglaia elaeagnoidea* and *Alseodaphne semecarpifolia* had poor recruitment. In general, understorey growth was dominated by shrubs (2483 per 0.3 ha) and not by tree saplings (562).

Discussion

Species richness

The Western Ghats harbour over 3500 species of flowering plants out of the 17,000 species described from India¹⁸. The Agasthyamalai range in the southern Western Ghats alone includes more than 2000 or 57% of the

3500 species⁷. Of these, 173 or 8.65% occurred in the transects at Kakachi.

The species-area relationship for the horizontal transects shows that by 4500 m² the species accumulation curve saturates for transects at 1200 m and 1300 m. For the transect at 1450 m, it did not saturate and is likely to increase even beyond the 50th plot (0.50 ha). This may be due to the transition in vegetation type at this altitude. The shape of the species area curves obtained at Kakachi was similar to those of Silent valley¹⁵ and Anamalais¹⁹ as most of the species there were also encountered by 0.45–0.5 ha. These cannot be strictly compared because they involve non-contiguous plots separated by long distances. Similar saturation of species area curves between 0.4 and 0.5 ha are also recorded from montane rainforests in South America²⁰. Further, the log normal distribution of species abun-

Table 4. Summary of vegetation studies in the evergreen forests of Western Ghats

Locality	Vegetation type	Altitude (m)	Plot (m ²)	No. of trees	Basal area (m ²)	No of species	Shannon index (H')	Source
1 Silent Valley Kerala	<i>Palaquium ellipticum</i> <i>Cullenia exarillata</i> <i>Ficus</i> sp.	1120	800		102.7	34	4.08	22
2 Silent Valley Kerala	<i>Elaeocarpus tuberculatus</i> <i>Eugenia</i> sp. <i>Poeciloneuron indicum</i>	1050	500		47.7	36	4.15	22
3 Attapadi Kerala	<i>Cullenia exarillata</i> <i>Mesua ferrea</i> <i>Palaquium ellipticum</i>	1100	1000			37	3.52	22
4 Kadamakal N. Coorg Karnataka	<i>Dipterocarpus indicus</i> <i>Kingiodendron pinnatum</i> <i>Humboltia brunonis</i>	650–700	1600	2926	64.9	70	4.3	15
5 Naravi Karnataka	<i>Dipterocarpus indicus</i> <i>Humboltia brunonis</i> <i>Poeciloneuron indicum</i>	750	1600	1925	70	35	3.6	15
6 New Someshwara Karnataka	<i>Dipterocarpus indicus</i> <i>Diospyros candolleana</i> <i>Diospyros oocarpa</i>	420	1400	1314	67.7	25	3.2	15
7 Magod Karnataka	<i>Persea macarantha</i> <i>Diospyros</i> sp. <i>Holigarna arnottiana</i>	370	1600	1875	67.7	35	4	15
8 Attapadi Kerala	<i>Cullenia exarillata</i> <i>Mesua ferrea</i> <i>Palaquium ellipticum</i>	900	2000	1520	59.6	32	4	15
9 Suthanbi	sub type 1 (of Attapadi)	750	1600	1200	60.7	32	3.7	15
10 Bhagawati	sub type 2 (of Attapadi)	900	1000	2250	64	12	2.1	15
11 Kankumbi Karnataka	<i>Memecylon umbellatum</i> <i>Syzygium cumini</i> <i>Actinodaphne angustifolium</i>	780	1600	1787	40.3	44	4.1	15
12 Sengaltheri Kalakad Tamil Nadu	<i>Cullenia exarillata</i> <i>Mesua ferrea</i> <i>Palaquium ellipticum</i>	900–1170	1000	852 856 915 725 885 574	59.73 77.38 55.3 94.64 64.87 61.7	77 64 85 84 82 80	3.5 3.3 3.6 3.6 3.7 3.6	14 14 14 14 14 14
13 Puthuthottam cardamom plantation Anamalais Tamil Nadu	<i>Cullenia exarillata</i> <i>Mesua ferrea</i> <i>Palaquium ellipticum</i>	1085	1600	812		31	4	19
14 Varagaliar RF Anamalais Tamil Nadu	Transition between <i>Cullenia exarillata</i> <i>Mesua ferrea</i> <i>Palaquium ellipticum</i> and <i>Dipterocarpus bourdilonii</i> <i>D. indicus</i> <i>Anaclosa densiflora</i>	650	1600	1063		41	4.8	19
15 Nelliampathy Kerala	<i>Palaquium ellipticum</i> <i>Cullenia exarillata</i> <i>Mesua ferrea</i>	950	10,000	496	61.9	30	1.28	24
16 Kakachi Kalakad Tamil Nadu	<i>Cullenia exarillata</i> <i>Aglaia elaeagnoidea</i> <i>Palaquium ellipticum</i>	1250–1450	38,200	582.7	42.03	90	4.87	Present study

Singh *et al.*²² and Pascal¹⁵ considered plants > 10 cm GBH, Parthasarathy *et al.*¹⁴, > 30 cm GBH and others > 10 cm DBH.

dance at Kakachi shows that only 7 species could be encountered with additional sampling. Extensive random search in the area revealed 6 uncommon species which were not encountered in the plots. Therefore it appears that a linear plot of 500 m × 10 m (0.5 ha) is sufficient to estimate the diversity of tree species at Kakachi, provided the elevation and the vegetation type remains the same. This may also be true for other mid-elevation forests of the Western Ghats.

In terms of number of tree species per unit area, Kakachi forests appear to have the highest density. Although we did not sample a sufficiently large contiguous area for the work reported here, our ongoing studies for three 1 ha plots reveal on an average 45 species of >10 cm DBH per hectare. Comparable data from similar undisturbed sites are not available. Studies by Pascal^{15,21} and Singh *et al.*²² in the Western Ghats calculated species richness and diversity at 10 cm GBH (3.3 DBH) or 40 cm GBH (12.7 DBH) which are not on par with the present study (see Table 4). Removal of girth limits also does not allow for comparison because of smaller sampling area of Pascal¹⁵ (0.2 ha) and Singh *et al.*²² (<0.1 ha). When similar comparisons are made with neotropical forests, Kakachi with 45 species per hectare is less species rich than BCI in Panama (176 spp. per ha); Upper Amazonia (155–283 spp. per ha) and La Selva in Costa Rica with 100 spp. from 2 to 4 ha (ref. 23). However it should be noted that all these are low-land rainforest sites having greater richness than montane sites.

Shannon index of diversity (>10 cm DBH) for Kakachi appears to have a higher value than other sites in the Western Ghats such as Anamalais¹⁹, Nelliampathy²⁴ and Sengaltheri, Kalakad¹⁴. Differences in the computations of Shannon index could make comparisons difficult. For instance, the Shannon value at Kakachi calculated to base 'e' is only 3.37 while that to base 2 is 4.87. Magurran²⁵ suggests the use of the latter for all purposes. Further, because of differences in area sampled, lack of uniform plot dimensions and standard girth or diameter classes, it is difficult to compare sites. Overall, Kakachi appears to be the most diverse site of all the wet evergreen forests sampled in the Western Ghats. The possible reasons for high diversity at Kakachi could be the larger area sampled and the linear nature of the transects which better estimates species diversity. Moreover the transition in vegetation type at 1450 m from the tropical wet evergreen to subtropical evergreen forest contributed to higher species richness. Further, bioclimatic and topographic factors like bimodal rainfall regime and relatively steeper slopes at Kakachi compared to other sites could also be a cause for some of the differences in species richness observed.

The vegetation of Kakachi does not fit the classical *Cullenia Mesua Palaquium* series described by Pascal¹⁵.

Attapadi forest of Kerala, which also has a similar dominance of *Cullenia Aglaia* and *Palaquium*, and where *Mesua* also occurs as one amongst the 6 dominant species, is identified as the *Cullenia Mesua Palaquium* series by Pascal¹⁵. In the Kakachi forest *Mesua* does not occur at all and hence can be described as *Cullenia Aglaia Palaquium* type which can be considered as a new subtype of *Cullenia Mesua Palaquium* series. However the *Cullenia Mesua Palaquium* type occurs only at a lower altitude (1000 m), contiguous with the Kakachi forest.

The flora at Kakachi consists of 50.6% tree species (>10 cm DBH), 29.8% shrubs, 10.7% herbs and only 8.9% were lianas. Based on floras, Daniel *et al.*²⁶ have shown that for the Western Ghats in general, herbs (52%) form the dominant component followed by trees (20%) and shrubs (16%). Similar comparison with other sites in Neotropics like BCI, La Selva and Ecuador also reveals domination by herb and shrub community, with less than 25% belonging to trees of >10 cm DBH^{23,27}. The overwhelming dominance of tree flora in Kakachi appears similar to the Manus reserve in Amazonia^{23,28}. This may be because in Kakachi like Manus the epiphytes and herbs were not completely surveyed, which could increase the floristic diversity, thereby reducing the dominance of tree and shrub species.

Stem density and basal area comparisons are again possible only for a few sites as mentioned before where values pertain to 10 cm DBH. Among them, Kakachi with 582.7 stems per ha, has a lower value than sites in Sengaltheri of Kalakad (Table 3) and evergreen forests of Karnataka²⁹. Some sites at Sengaltheri were located in 30-year-old abandoned cardamom plantations which had larger number of small-sized stems leading to higher stem density per unit area¹⁴. Basal area comparison reveals that the values in Kakachi are the lowest among other evergreen forests in the Western Ghats. For sites like Nelliampathy²⁴ and Sengaltheri¹⁴, higher basal area might be due to the differences in the altitude sampled. Both these sites were between 1000 and 1200 m altitude representing very tall and large diameter trees. Species composition also contributes to this difference as some species like *Mangifera indica* and *Bischofia javanica* which are restricted to this altitude have very large girths (>3 m) (personal observation). Exceptionally high basal area recorded in Silent Valley sampled by Singh *et al.*²² (Table 4) may be due to smaller plot size which allows the presence of only one or two large trees¹⁵.

Endemism

The Agastyamalai range is known for high levels of endemism in plant species¹⁰. Of the 2000 species found in Agastyamalai (2000 sq km), roughly 7.5% are localized endemics. At Kakachi, of the total 173 plant spe-

cies found in the transects, 8 (4.62%) were localized endemic and account for 5.33% of the total endemic species in the region. Of these, trees account for 5 (62.5%), shrubs 2 (25%) and lianas 1 (12.5%).

Herbs did not have any endemics. This indicates that trees in the evergreen forest make a substantial contribution to the levels of endemism in this area. Moreover, Pascal²¹ also refers to high levels (43.4%) of endemism among trees and shrubs in the *Cullenia Mesua Palaquium* forest. Much of this can be attributed to the overall prevalence of endemism among tree species in the hill top floras of the Western Ghats³⁰.

Elevational aspects

The effect of small scale altitudinal changes on species richness in the Western Ghats is not well documented. In Kakachi, transects and the subplots demonstrate that species richness increased with elevation. This increase is due to the changes in vegetation type toward the ridge from the wet evergreen forest to a more tropical sub montane forest³¹. Some species such as *Alseodaphne semicarpifolia*, *Xanthophyllum flavescens* and *Syzygium densiflorum* are common only above 1400 m. Many species like *Tricalysia apiocarpa*, *Drypetes longifolia* and *Elaeocarpus munronii* have a more disjunct distribution within the 250 m altitude range. Some species like *Palaquium ellipticum* were restricted to lower elevations and only one half of the tail was sampled in this study. The other half of the distribution occurs below 1200 m which was not sampled. Some of the elevational distribution patterns are probably due to edaphic factors and influence of strong winds especially around the exposed areas and presence of *Ochlandra* spp in the study plot. Some species like *Dimocarpus longon*, *Canarium strictum* and *Heritiera papilio* were rare in the site because their optimal habitats occur at lower elevations where they are common. More sampling from lower elevations is required to determine the complete elevational ranges of these species.

Regeneration

Though many forests in the Western Ghats are reported to have poor regeneration^{4,5}, the first five dominant species at Kakachi show adequate regeneration ranging from 7 to 135 individuals per 0.1 ha at the 1 to 10 cm DBH levels. Similarly in BCI it ranged from 4 to 47 individuals per 0.1 ha of the most abundant tree species³². However low density species like *Syzygium mundagam* and species with large diameter classes like *Calophyllum austroindicum* did not have many saplings in the plots. These species also suffer very high mortality at the seed stage from vertebrate seed predators³³.

Such high predation could have depressed their recruitment. Regeneration in such species could also be episodic.

Conclusion

The wet evergreen forests of Kakachi show a high level of plant species diversity compared with the other evergreen formations in the Western Ghats. It also shows good regeneration relative to many dry forests in India.

Comparative analysis with other sites in the Western Ghats was severely hindered due to lack of any standard protocol in sampling vegetation. Future studies should follow certain norms of standardized sampling which could be readily used across sites for easy comparison. This is very important for sites which will be used for monitoring vegetation dynamics.

Even though this medium elevation forest is one of the least disturbed sites in the Western Ghats, its biodiversity is threatened due to fragmentation of climax forest. Fragmentation has resulted from anthropogenic pressures like tea and cardamom plantations and other developmental projects like dams and reservoirs. Though the KMTR is spread over 887 sq km, the evergreen forests are restricted to a much smaller area at the medium elevations which are also the preferred sites for tea, coffee and cardamom plantations. Further, these forests in the Agasthyamalai hills serve as watershed areas for many major perennial rivers like Tambaraparani, Manimuthar, Kodayar and Pachaiyar which are the main water source for the southern districts of Tamil Nadu. Thus preservation of these forests is crucial not only for maintaining the biodiversity, but also for meeting the basic needs of the human populations in the plains.

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Managing the impact of seasonal rainfall variability through response farming at a semi-arid tropical location

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A method of Response Farming Programme for managing risks associated with variable seasonal rainfall was developed for a semi-arid location using daily rainfall records for the period 1971-94. The risks are intense rains threatening soil erosion, prolonged heavy rains threatening water logging, prolonged low rainfall periods, early cessation of rains long before the maturity of crops and too little rainfall in relation to crop water requirements. Onset relations, i.e. relations between season rainfall parameters (amount, duration and average rainfall per day) and date of onset of the rainy season are determined. It has been demonstrated how these relationships can be used in the selection of crops/cropping systems, fertilizer application rates, plant population, etc.

In rainfed agriculture, farmers must cope with rainfall variability both within and between seasons. If rainfall

were uniform every year, farmers would choose a single management plan. Crops, planting date, seeding rate, fertilizer and insecticide would be planned for that anticipated single rainfall pattern. Obviously, this is not the case and variability of rainfall creates major problems. Response Farming is a means of coping with this seasonal rainfall variability which provides a method of identifying and quantifying seasonal rainfall variability and its related risks and of addressing the latter at the farm level. This is accomplished through improved prediction of expected rainfall behaviour in the approaching cropping season enabling improved decisions at the field level. The date of onset acts as a rainfall (amount, duration, average rainfall per day) predictor for the remainder of the season. Current rainfall is used to determine the management strategies which are responsive to the weather patterns. Stewart¹ developed Response Farming Strategies by studying the relationship between

onset of the rainy season versus duration of the rainy season and onset versus rainfall amount during the rainy season for two locations, Niamey and Bouza. In order to assess the rainy season potential for crop growth, Siva Kumar² has determined the relationship between duration and onset for 58 locations in the Sahelian and Sudanian Zones of Niger and Burkina Faso. In the present study, two types of analysis were done. The first analysis provides a general characterization of historical rainfall behaviour for purposes of determining cropping potential in new development areas or presently farmed lands. Analysis two simulates historical water supply conditions for crops of specified types and maturities. This type of analysis assumes that final decisions are based on date of onset but the effective cropping season begins at germination.

Materials and methods

The daily rainfall data for the period 1971–94 recorded at Hayatnagar Research Farm of Central Research Institute for Dryland Agriculture (CRIDA), Hyderabad (17°27'N, 78°28'E) were utilized in the present study. The farm situation represents semi-arid tropical region of Peninsular India, with an average annual rainfall of 733 mm and potential evapotranspiration of 1754 mm. Nearly 70% of the total precipitation is received during the southwest monsoon season (June to September). Post-monsoon rains are not uncommon and occur around 18% of average annual rainfall. The monthly normal potential evapotranspiration values given by Rao *et al.*³ were utilized in the present study.

Soils of the experimental site are shallow alfisols. Moisture storage in the profile is only 24% by volume and permanent wilting point is 14% by volume. The soils are low in nitrogen and extremely low in phosphorus. The yields of sorghum (CSH-6), pearl millet (BJ-104), castor (Aruna), pigeonpea (HY-2) and sunflower (68415) were simulated (1971–94) using the water production functions developed by Victor *et al.*⁴

The following assumptions were made to identify beginning, end and duration of the rainy season to characterize the rainy season behaviour^{5,6}. The date of onset of rains is defined as that date after 28 May, when rainfall accumulated over two consecutive days is at least 20 mm. The date of ending of the rains is taken as that date after 1 September following which no rainfall occurs over a period of 30 days. The duration of the rainy season is taken as the difference between beginning and ending of rainy season. Frequency distribution of dates of beginning and ending of the rains and duration of the rainy season were estimated by fitting a normal distribution. Then the probabilities for different durations of the rainy season for early, normal and delayed onset of rains were obtained.

A computer-based Response Farming Programme which incorporates a bare soil water balance algorithm⁷ was developed to simulate historical water supply conditions based on which the date of onset, germination and duration of the crop growing season were identified. The following are the inputs to this analytical programme.

- Daily rainfall (mm).
- Mean monthly potential evapotranspiration (PET) (mm).
- Daily PET values were calculated/assumed as follows^{8,9}:

$$\text{PET} = (\text{Mean monthly PET}) * 1.1/30$$
 on days with no rainfall
 4 mm/day on days with 1–10 mm rainfall
 3 mm/day on days with 11–20 mm rainfall
 2 mm/day on days with > 20 mm rainfall.
- Field capacity of the soils of the experimental site is 0.24 by volume.
- Permanent wilting point is 0.14 by volume.
- Air dry water content is 0.04 by volume.
- 1 May to begin search.
- First accepted date of onset for cropping, 1 June.
- *Onset criterion*: 20 mm of stored water in the 30 cm soil profile from the new rains. Evaporation assumed to continue throughout the soil–water accumulation process. Losses to runoff assumed zero.
- Planting to begin after a minimum delay of three days provided the rainfall is less than 5 mm/day.
- *Germination criterion*: 15 mm of water stored in the 30 cm soil profile to effect germination. Usually occurring on the first day following planting.
- Last search date for season end rains is the crop maturity date.
- *Final rain date criterion* is to sum rainfall backward from the maturity date to accumulate 50 mm, then proceed forward toward the maturity date until average daily rainfall fell below 2.5 mm/day.

The analysis was done for 90, 100 and 150 days maturity crops which corresponds to sorghum [(CSH-6) and pearl millet (BJ-104)], sunflower (68415) and [pigeonpea (HY-2) and castor (Aruna)] respectively.

Results and discussion

General characterization of rainy season behaviour

Quantification of risks due to variable seasonal rainfall. The following risks, viz. (a) rainfall intensity, (b) prolonged heavy rainfall periods, (c) prolonged low rainfall periods were quantified. The following conclusions were drawn from the analysis.

Intense rains threatening erosion of the basic soil resource: Based on the frequency distribution of daily rainfall data, the chance of occurrence of 75 mm or

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more is very low (0.5%) and the probability of getting the rainfall in the range 50–75 mm per day is only 0.3%. However, the occurrence of low rainfall amounts (<25 mm/day) is very high (98%). Considering the analysis for shallow alfisol, it was judged that the erosion hazard in the present rainfall circumstances is moderate.

Prolonged wet and dry spells: A first order Markov chain probability model was fitted to daily rainfall data to calculate initial and conditional probabilities for two rainfall limits (2.5 mm and 10 mm)^{10,11}. Using these probabilities of dry and wet spells and, conditional dry spells, which is the length of a dry spell conditional on the day prior to the beginning of the period being rainy, were calculated and are given in Table 1. These data are presented from 3rd week of May to last week of October (21–44 standard meteorological weeks) which is the period during which rains are received. These data provide quick overview of risks due to dry spells during the rainy season.

It is evident from the analysis that wet spells in the current cropping season exceeding 10 mm per day continuously for five days or more in a week are infrequent (<1%). It is seen from Table 1 that mid-season dry spell during August (32–35 weeks) exceeding five days is in the range of 16–35%. The conditional dry spell length five days during 23–26 week is in the range of 12–25%. However, if the onset is earlier than 23rd

week, the probability of getting conditional dry spell of five days increases (more than 46%), which indicates that sowing field crops with first rains before 23rd week is fraught with considerable risk while the period after 23rd week is slightly favourable since the probability of dry spell decreases rapidly, indicating significantly reduced risks to the emergence and subsequent growth of crops.

Relationship between date of onset and rainfall amount and date of onset and rainy season duration. Seasonal rainfall amount, duration and average rainfall per day are linked to the date of onset. For predictive purposes, onset was divided into three distinctive time periods, viz. early (May 28–June 10), middle (June 11–30), and late (July 1 onwards).

Table 2 shows the median values of monsoon rainfall attributes at Hayatnagar Research Farm for the entire 24-year record studied and then for each of the three groupings of seasons defined by the prediction criteria outlined above. It is evident from Table 2 that over all median cropping season rainfall of 581 mm persisting 150 days with an average rainfall of 4.5 mm per day. Seasons with early onset show higher rainfall amount, longer duration and higher average rainfall per day. Seasons with middle period onset have lower rainfall, same duration and less average rainfall per day. Late onset seasons have the least rainfall and shortest durations, but paradoxically the highest average rainfall per day of all. The importance of the differences among the predicted groupings in Table 2 is more easily grasped when ranges of values within each of the categories are seen in addition to the median values. Table 3 presents the ranges, starting with the 24-year record as if no predictions were involved. Rainfall amount ranged from low of 327 mm to a high of 1077 mm. Duration is 79 to 167 days and average rainfall per day is as low as 2.2 mm per day and as high as 6.8 mm per day. If the onset is early (by June 10), the rainfall was never less than 395 mm but fell to 327 mm with middle period onset and to 448 mm in the late seasons. Similarly, duration never fell below 120 days for the early onset seasons and decreased to 92 days for middle period onset and 79 days for late onset seasons. The average rainfall per day for the early and middle onset periods ranges from 2.2 to 6.8 mm per day. However, the late onset seasons show high average rainfall per day.

Selecting crops/cultivars and cropping systems based on predicted duration. Actually, crop/cultivar selection based on predicted duration requires consideration of (a) length of growing season (maturities) of different crops and cultivars in the planning site environment, (b) rapidity of planting (no. of days it takes to plant following onset), (c) soil depth and water-holding

Table 1. Probabilities (%) of dry spell and conditional dry spell lengths during different weeks at Hayatnagar, Hyderabad

Standard meteorological week	Dry spell (days)			Conditional dry spell* (days)		
	> 3	> 5	> 7	3	5	7
21	79	67	58	85	73	62
22	65	49	36	61	46	34
23	57	39	27	54	37	25
24	42	23	13	38	21	12
25	58	41	28	38	26	18
26	55	37	25	35	24	16
27	35	18	9	26	13	7
28	49	31	19	41	25	16
29	51	33	21	32	21	13
30	45	26	15	33	19	11
31	39	21	11	34	18	10
32	42	24	13	31	18	10
33	34	16	8	24	12	6
34	54	35	23	49	32	21
35	49	31	19	37	23	15
36	64	48	36	46	34	25
37	58	40	28	43	30	21
38	50	31	20	27	17	11
39	60	43	30	42	30	21
40	52	33	21	33	21	14
41	70	56	44	41	32	25
42	83	73	65	54	48	42
43	72	58	47	40	32	26
44	71	57	46	44	35	28

*Calculation of dry spell conditional on the day before the start of the period under consideration being rainy.

capacity, (d) crop coefficients for the estimation of water requirements, and (e) evaporative rates through the season.

The goal of planning exercise is to select crop/cultivars that will reach maturity within the rainy period or within a time period following the final rain date but before completely running out of extractable soil water.

For example, from the data in Table 4, it is seen that in 38% of years only more than 150 days duration is possible if the onset is normal. However, this length of the season does not support sequence cropping even with short duration crops and varieties as the soil has low water-holding capacity. The long duration crops like castor or pigeonpea occupy complete growing season. Any intercrop reduces the yield of castor. With short duration cereal crops like sorghum and pearl millet, quite a good length of growing season is left unutilized. Therefore intercropping of sorghum/or pearl millet with pigeonpea is the best system suited for this region.

Characterization of rainy season behaviour with reference to specific crops. The analysis was done for 90, 100 and 150-day crops. The relationship between rainy period duration and date of onset, average crop season rainfall and date of onset, and total rainfall in 30 days after germination and date of onset are presented for

sorghum and castor in Figures 1 and 2. The following important conclusions can be drawn from these figures.

Rainfall duration of 80 and 110 days or more is sufficient to permit a 90 and 150 days sorghum and castor cultivars respectively to produce yields greater than 75% of the maximum. Both sorghum and castor can be seeded till the end of July. However, if sorghum is seeded beyond June, its yields will be reduced drastically due to shootfly attack. As an alternative, pearl millet can be seeded up to mid-July. With later seedings, this crop matures in cool weather and results in ergot infection. Under delayed seeding conditions up to the end of August, minor millets like finger millet, foxtail millet can be seeded. As a last chance horsegram can be seeded up to the end of September.

Following determinations of cultivar maturities to be grown, the next selection is type of crops themselves. This is done by relating average season rainfall to daily water requirements of crops of interest. Average season rainfall per day is calculated by dividing the total season rainfall by the maturity of the crop. For example, for sorghum, it is seen from Figure 1 b that average season rainfall per day ranges from just above 1.9 mm per day to just below 8.0 mm/day. Season water supply averaging at or above 5 mm/day should be capable of producing yields greater than 75% of maximum provided the

Table 2. Median values of monsoon cropping season onset dates and rainfall amount, duration and average rainfall per day

No. of years	Onset period	Monsoon rainfall			
		Onset date	Amount (mm)	Duration (days)	Avg. ppt. (mm/day)
<i>Median values</i>					
24	All onsets (May 28–July 13)	6–13	581.0	150	4.5
<i>Three groupings of seasons</i>					
10	Early: By June 10	6–13	706.0	152	4.75
13	Middle: June 11–30	6–15	540.0	150	3.70
1*	Late: July 1 onwards	7–13	448.0	79	5.70

* Actual values; Avg. ppt. = Average precipitation.

Table 3. Ranges of monsoon cropping season onset dates and rainfall amount, duration and average rainfall per day

No. of years	Onset period	Monsoon rainfall		
		Amount (mm)	Duration (days)	Avg. ppt. (mm/day)
<i>Ranges of values</i>				
24	All onsets (May 28–July 13)	327–1077	79–167	2.2–6.8
<i>Three groupings of seasons</i>				
10	Early: By June 10	395–1077	120–165	2.8–6.8
13	Middle: June 11–30	327–874	92–167	2.2–6.5
1	Late: July 1 onwards	448	79	5.7

Avg. ppt. = Average precipitation.

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fertility is not lacking; competing weeds, pests and diseases are controlled and water losses are minimal. 50–75% of the maximum yields can be expected until

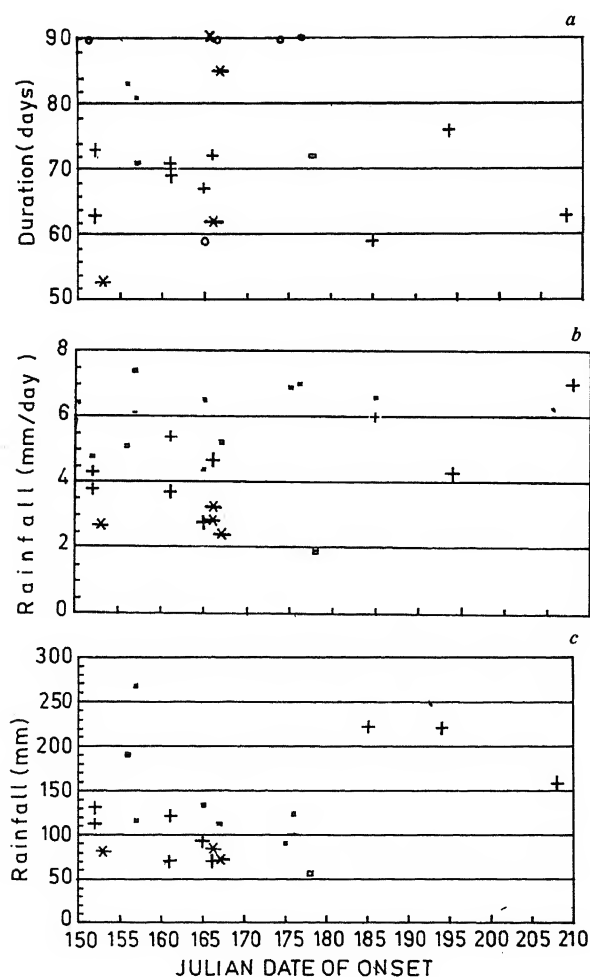
Table 4. Probabilities (%) of growing season length exceeding specified durations for different dates of onset of rains at Hayatnagar, Hyderabad

Date of onset	Length of the growing season (days) exceeding			
	90	100	130	150
23 May	100	100	100	76
2 June	100	99	89	58
12 June*	100	96	76	38
22 June	99	89	58	21
2 July	96	76	38	9

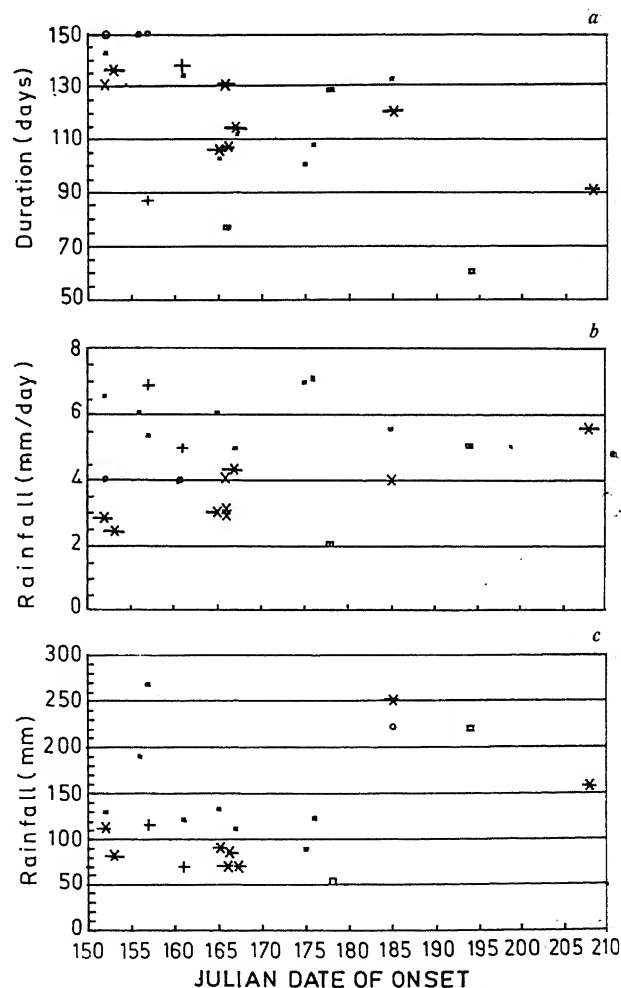
*Mean date of onset of rains.

the average daily water supply falls to about 4 mm after which subsistence level production is expected (50–25% of the maximum yields). When the daily water supply falls below 2 mm, the yields less than 25% of the maximum can be expected.

The relationship between total rainfall in the next 30 days after germination and date of onset enables the farmers to take decisions for the second time in deciding fertilizer rates and plant population. Here, it is assumed that water supply up to 30 days after germination is adequate to provide low risk guidance on whether to add fertilizer for high rainfall conditions or to thin and reduce the plant populations for low rainfall conditions. For example, it is evident from Figure 1 c that if total rainfall in 30 days after germination exceeds 100 mm, sorghum yields greater than 75% of the maximum can



Sorghum Yields: ■ >75% + 75-50% * 50-25% □ <25%



Castor Yields: ■ >75% + 75-50% * 50-25% □ <25%

Figure 1 a-c. a, Relationship between rainy period duration and date of onset at Hayatnagar; b, Relationship between average crop season rainfall and date of onset at Hayatnagar; c, Relationship between total rainfall in the next 30 days and date of onset at Hayatnagar.

Figure 2 a-c. a, Relationship between rainy period duration and date of onset at Hayatnagar; b, Relationship between average crop season rainfall and date of onset at Hayatnagar; c, Relationship between rainfall in the next 30 days and date of onset at Hayatnagar.

be expected. Therefore, if water supply in 30 days after germination exceeds 100 mm, apply fertilizers and if less than 100 mm, reduce the plant population by thinning and leave the fertilizer as before. Actually the 30 day water supply in most years is far above the 100 mm minimum which signals yields greater than 75% of the maximum. This means that the 100 mm rainfall is often reached long before the 30 days have passed. The decision, therefore, for additional nitrogen fertilizer can usually be made well before the action must be taken, thus extending preparation time. The predictive criteria, decisions and recommended actions suggested in this paper are not definitive. These are yet to be tested at field level before using them for operational purpose.

Conclusions

Seasonal variation in rainfall poses the greatest climatic risks to agricultural production in rainfed farming areas. In the variable rainfall zones at least five aspects of seasonal rainfall behaviour need to be evaluated in terms of agricultural production. These are intense rains threatening erosion, prolonged heavy rains threatening water logging, prolonged low rainfall periods within the cropping season, early cessation of rains too long before crop maturity or too little rainfall in relation to crop water requirements. A number of relationships between the onset of cropping season rainfall and such farm relevant rainfall parameters, such as rainfall amount, duration and average rainfall per day are determined by analysing the historical rainfall records. cursory examination of the rainfall data indicates that risk from prolonged heavy rains is not much, particularly on shallow alfisols. But spells of dryness or exceedingly low rainfall do pose risk during the cropping season. Duration is very much correlated with date of onset of the rainy season, hence is the key factor in selecting maturities of crops/cultivars and cropping systems to be grown. Relating duration to onset dates provides season-by-season guidance. Following determination of cultivar maturities to be grown, the next selection is the type of crops themselves. This is done by relating

average water supply per day to daily water requirements of crops of interest. The crux of the management problem with variable rainfall condition is to adopt soil fertility and plant populations to actual rainfall conditions in the season at hand. Fortunately farmers can delay final decisions on these questions until about 30 days after germination. In the case of sorghum if water supply in 30 days after germination exceeds 100 mm, apply fertilizer and if less than 100 mm, reduce plant population by thinning and leave fertilizer as before.

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Ultrasonic agitation of Kevlar fibres

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Kevlar 49 fibres exposed to ultrasonic waves suffer axial compression manifested by the introduction of kink bands, dispersion of fibrils and macrobuckling. Application of subsequent tensile load, however, influences the kink bands.

In polymer research, ultrasonic waves are traditionally used for the study of dynamic processes such as polymerization and degradation. Nondestructive testing of resins with ultrasonic waves is also well known. Reviews describing different types of studies on polymeric materials employing ultrasonic waves are available^{1,2}. In this communication we present details of some observations concerning the residual effects of exposure to ultrasonic waves on the aramid fibre Kevlar.

A bundle of Kevlar 49 fibres (approximately 150 mm long and 2 mm thick) made commercially available by DuPont Inc. USA, was exposed to ultrasonic waves of frequency 40 ± 3 kHz generated by a fully transistorized ultrasonic generator. Distilled water was used as the transmitting medium. Figure 1 is a schematic representation of the experimental arrangement used. The generator and the tank containing the sample were coaxially connected. Fibres were subjected to cumulative exposure to ultrasonic waves for 1, 3 and 6 h respectively. The choice of the time intervals was purely arbitrary. In the course of the agitation, the distilled water surrounding the sample was getting heated, the temperature rising to $\approx 40^\circ\text{C}$ after ≈ 2 h of agitation. At such stages, the agitation was disrupted and continued further only after the liquid returned to the ambient temperature of $\approx 25^\circ\text{C}$. Surface characteristics of ultrasonically agitated filaments were subsequently examined in an optical microscope using polarized light in the reflection geometry.

The most conspicuous effect of ultrasonic agitation on Kevlar fibres is the introduction of kink bands. Figure 2 compares the surface characteristics of Kevlar fibres both prior to and after ultrasonic agitation. Fibres agitated for 1 h include isolated, faint kink bands which are either normal or inclined to the fibre axis. In contrast, fibres agitated for 3 and 6 h exhibit a concentration of V- and X-shaped bands which are distinctly darker and more in number than the bands found in fibres agitated for 1 h. The V- and X-shaped bands are found almost continuously along the length of the agitated fibre. Kink bands, as is well known, correspond to structural deformation³. The bands which are inclined to the fibre length are attributed⁴ to tangential deformation of the

radially oriented molecular architecture⁵ which characterizes Kevlar fibres and the perpendicular bands are correlated with radial splitting of the fibrils⁴. The V- and the X-shaped features represent multiple bands and they result from overlapping inclined and normal kink bands. The surface characteristics of Kevlar thus indicate that exposure to ultrasonic waves introduces structural deformations which in turn lead to the formation of kink bands and the severity of the deformation increases with increase in the duration of exposure.

Ultrasonic agitation is also found to cause dispersion of fibrils (Figure 3 a). The separated fibrils tend to get entangled around the main fibre with subsequent agitation (Figure 3 b). Figure 3 c shows another feature, viz. the macrobuckling. Along the length of the agitated fibre, notch-like regions of the type shown in Figure 3 c where fibre changes direction or buckles, have been observed. Conspicuously, the macrobuckling is less rampant than the formation of kink bands and also the dispersion of fibrils.

It must be pointed out that the formation of kink bands, separation of fibrils and macrobuckling are characteristics typical of axial compression of Kevlar fibres⁶⁻⁸. The remarkable similarity between the characteristics of the ultrasonically agitated and the axially compressed Kevlar fibres suggests that exposure to ultrasonic agitation causes axial compression. Occurrence of such axial compression is not surprising because the fibres were positionally unconstrained during the process of ultrasonic agitation. It is not unlikely that at some point of time they got favourably oriented to suffer axial compression by the continuously impinging ultrasonic waves.

The kink bands, both single as well as multiple, formed by ultrasonic agitation of Kevlar are found to be sensitive to subsequent tensile loading. The effect of tensile loading was followed by using a spring loaded

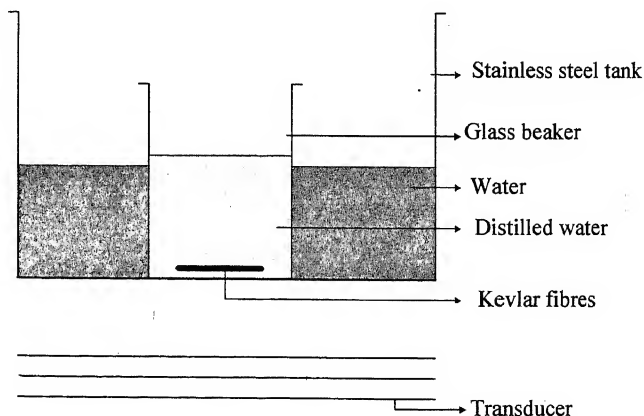


Figure 1. Schematic representation of the arrangement used for ultrasonic agitation.

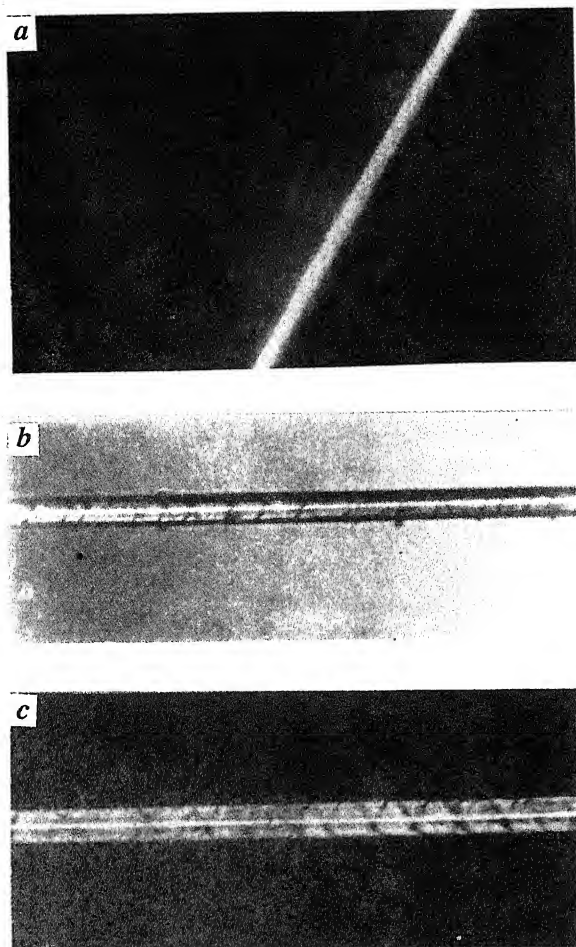


Figure 2 a-c. *a*, Surface of the $\approx 11\ \mu\text{m}$ thin fibre prior to ultrasonic agitation; *b* and *c*, V- and X-shaped kink bands in fibres agitated for 3 and 6 h respectively.

device of the type shown in Figure 4. In this device the fibre held taut between the two sets of screws could be stretched to different extents by adjusting the lead screw, S. It was found that with increase in the extent of stretching, the kink bands on the surface of the fibre turned progressively less intense and eventually, at some stage of stretching, the kink bands were not observable. It must be emphasized that on releasing the tensile load on the fibre, the kink bands did not reappear. These observations suggest that the structural deformations which led to the occurrence of kink bands are not permanent and the severity of these deformations could be lessened or removed with subsequent application of tensile load to the fibre. In order to test whether the kink bands which characterize fibres compressed by the recoil method⁸ also exhibit a similar behaviour, optical examination under the application of tensile load was extended to Kevlar fibres compressed by the recoil method. Interestingly, irrespective of the method of formation, under the application of tensile load, the kink

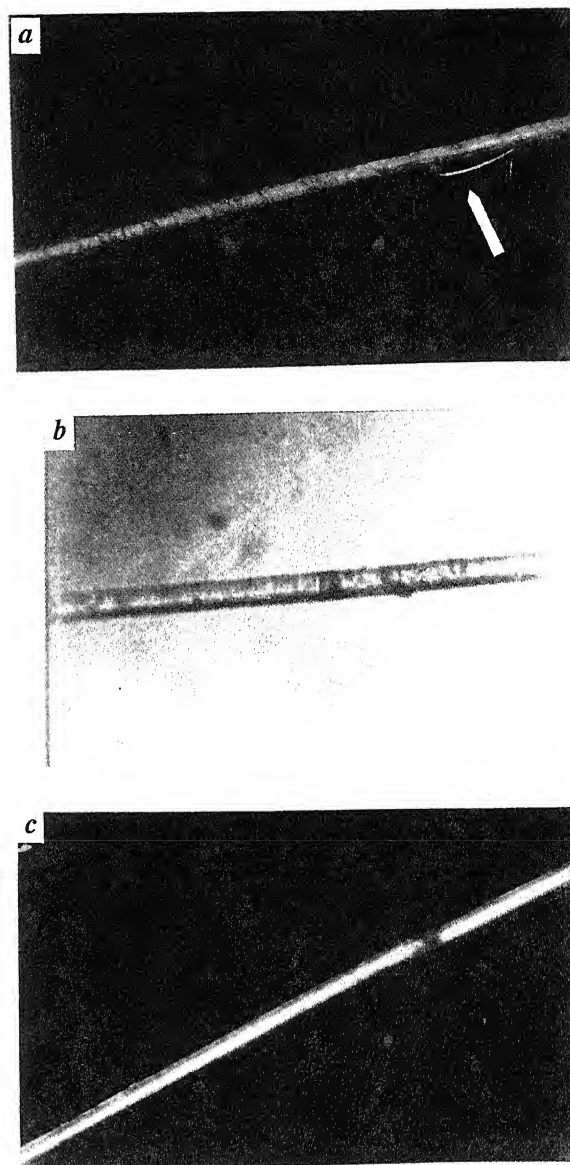


Figure 3 a-c. *a*, Dispersion of fibrils; *b*, Dispersed fibrils coil round the fibre; *c*, Macrobuckling of the fibre.

bands behaved in an identical fashion, viz. disappeared with the application of load. Figure 5 illustrates an example of the effect of stretching on kink bands. It must be pointed out that these observations pertain only to the surface of the fibre. Information on the effect of tensile load on ultrasonically induced deformations residing away from the surface and towards the core of the fibre is not available at the present moment. TEM observations are likely to provide insight into this aspect. It must be mentioned that in the present study, the stretching which the fibres were subjected to was not quantified. Consequently, calculations providing correlation between the tensile load and the energy needed for the removal of the deformation which caused the kink bands were not possible.

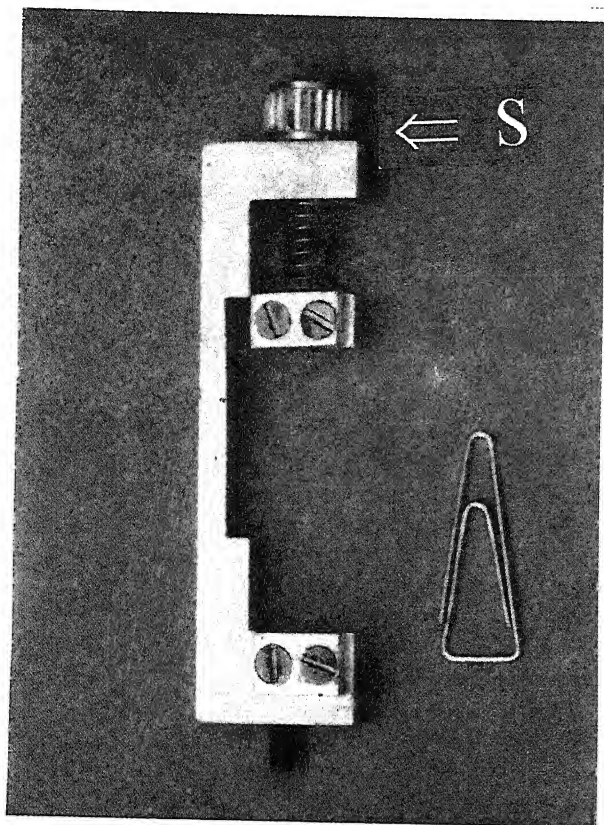


Figure 4. Device used for stretching the fibre.

In contrast with the kink bands, the macrobuckling introduced by ultrasonic agitation is not removed by subsequent stretching. The notch-like regions (Figure 3 c) representing change in direction thus appear to represent comparatively more severe and irreversible structural deformations introduced during ultrasonic agitation.

It must be mentioned that Allen and Roche⁹ have observed a similar effect of tensile load on Kevlar fibres. Their observations indicate that the pleated structure¹⁰ which characterizes Kevlar gets straightened with the application of tensile load. However, in contrast with the kink bands, the pleats reappear with the removal of the load.

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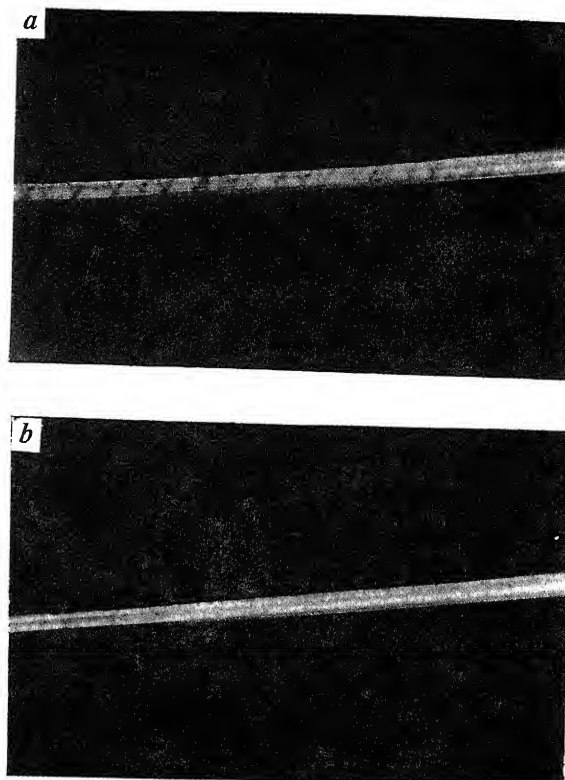


Figure 5. Kink bands: *a*, prior to and *b*, after stretching the ultrasonically agitated fibre.

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Spontaneous regeneration of pancreatic β -cells in EMC-D virus-induced diabetic mice and reversion from diabetic to normal state

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With an aim of developing a model of experimental diabetes by which spontaneous recovery and regeneration processes can be investigated, EMC-D virus-induced diabetic mice were used as a system to study regeneration *in vivo*. Ninety per cent of the EMC-D virus-inoculated mice developed diabetes within a week after inoculation as judged by the glucose levels in blood and urine. Fifty-five per cent of the diabetic mice died within a fortnight after virus inoculation. Thirty per cent of the total infected mice slowly recovered from the diabetic state as evidenced by diminishing glycosuria, hyperglycaemia and near normal histological architecture. The results indicate reversion from clinical diabetes to normal status. Thus EMC-D virus-induced diabetes in SJL/J mice provides a new model to investigate mechanism and factors involved in regeneration of pancreatic β -cells *in vivo*.

INSULIN-dependent (Type 1) diabetes mellitus is an autoimmune disease caused by the selective destruction of islet β -cells, and the arrest of their regeneration potential may lead to a total disappearance of the cells. At the onset of diabetes about 80–90% of the β -cells are destroyed, resulting in severe hyperglycaemia¹. Recently, it has been shown that viable and insulin-producing β -cells are present in human pancreatic islets at the onset of Type 1 diabetes². It is not known how the β -cells can be triggered to proliferate in response to a moderate loss, providing that the residual β -cell mass is sufficient to maintain the glucose homeostasis under the condition where the glucose tolerance is already impaired³. Few cases of spontaneous and long lasting recovery from severe insulin-dependent diabetes mellitus in human and from drug-induced diabetes in animals are known^{4,5}. It is not known whether β -cells in human beings regenerate. Since it is difficult to estimate regenerating β -cells in human cases of diabetes, it was thought worthwhile to use animal models of diabetes for this purpose. In the murine model of virus induced, insulin-dependent diabetes mellitus (IDDM), it has been shown that D-variant of EMC virus infects and lyses pancreatic β -cells⁶. The severity of diabetes is related to the extent of β -cell damage⁷. The earlier work on EMC-D induced diabetes in mice pointed out survival of diabetic mice for several days after virus infection, exhibiting abnormal glucose tolerance^{6,8–10}.

However, detailed clinical and histological studies of the surviving population were not carried out. Hence, the present study was designed to ascertain whether EMC-D virus-induced diabetic mice exhibit regeneration potential and eventual reversal of diabetes.

The D variant of EMC virus (plaque purified from mouse heart passaged M variant) was used in all the experiments. The source and preparation of EMC-D virus are described earlier⁶. Virus pools were prepared from L929 cells, and the virus titre was determined by plaque assay on L929 cells¹¹.

Male SJL/J mice were obtained from the Jackson Laboratory, Bar Harbor, Maine and housed in the animal facility in the Health Sciences Centre, University of Calgary, Calgary, Alberta, Canada. Except when noted, 5-week-old mice were used. A total of 60 mice were divided into 2 groups, control group of 20 mice and experimental group of 40 mice respectively. EMC-D virus was injected intraperitoneally into the experimental group (with 0.2 ml/mouse containing 100–500 p.f.u.). The control group was inoculated with 0.2 ml of phosphate buffered saline (pH 7.2).

Beginning 3 days after infection with virus, glucose levels in the urine were measured every alternate day with Diastix (Ames Division, Miles Laboratories Ltd., Etobicoke, Ontario, Canada). Blood glucose levels were measured in blood from the retro-orbital venous plexus by use of glucose oxidase assay with *o*-dianisidine dihydrochloride as the indicator dye⁶. Nonfasting glucose levels in the blood were measured on 5, 10, 15, 20, 25, 35 and 45 days after infection. The mean nonfasting glucose level of 20 uninoculated SJL/J male mice was 158 ± 19 mg/dl. In these experiments, any mouse with nonfasting glucose level greater than 215 mg/dl (3 standard deviations (SD) above the mean) was scored as diabetic.

Insulin was extracted from pancreas of mice sacrificed on 5, 20 and 45 days after infection by acid ethanol method¹² and the insulin content of the pancreatic extract was measured by RIA¹³ and expressed as μ g/g of pancreas. Briefly, pancreas were disintegrated by ultrasonic procedure at 4°C in 0.5 ml acid-alcohol solution (75% ethanol and 1.5% 10 N HCl v/v). The homogenates were kept at -20°C until insulin was assayed by RIA.

At 5, 20 and 45 days after virus inoculation, several mice from control and experimental group were sacrificed and the pancreas were fixed in Bouin's fixative. Paraffin-embedded sections were stained with haematoxylin and eosin and examined to score presence or absence of insulinitis and normal islet morphology.

Data obtained are summarized in Tables 1–3. Table 1 shows the glucose levels of blood and urine at various days after virus inoculation and the incidence of diabetes in the 2 groups. Animals in the control group did not show signs of development of diabetes throughout the

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period of observation as indicated by absence of glucose in urine and blood glucose levels < 200 mg/dl at all the time points noted. However, in EMC-D virus inoculated group 36 out of 40 mice (90%) became diabetic within a week after virus inoculation, as revealed by the presence of glycosuria and hyperglycaemia (blood glu-

Table 1. Changes in blood glucose (mg/dl) and urine glucose in control (C) and EMC-D virus infected (EMC-D) mice at different post-inoculation days

Days after EMC-D inoculation	Group	Blood glucose (mg/dl)	Urine glucose (+ ve/- ve)	No. of mice
5	C	180 \pm 11	- ve	20
	EMC-D	470 \pm 18	++++	36
10	C	140 \pm 17	- ve	10
	EMC-D	468 \pm 16	++++	26
15	C	200 \pm 12	- ve	10
	EMC-D	430 \pm 7	++++	16
20	C	180 \pm 15	- ve	10
	EMC-D	472 \pm 10	++++	4
	EMC-D (R)	264 \pm 13	++	12
25	C	140 \pm 17	- ve	10
	EMC-D	380 \pm 6	+++	4
	EMC-D (R)	210 \pm 9	- ve	12
35	C	110 \pm 11	- ve	10
	EMC-D	400 \pm 12	++++	4
	EMC-D (R)	186 \pm 9	- ve	12
45	C	140 \pm 11	- ve	10
	EMC-D	390 \pm 8	+++	4
	EMC-D (R)	182 \pm 15	- ve	12

EMC-D (R) = Regenerating mice; ++++ = Strongly positive; - ve = Absence of sugar.

Table 2. Changes in wet weight of the entire pancreas in control and EMC-D virus inoculated diabetic and regenerating mice at different post-inoculation days

Days	Control group (mg)	EMC-D virus inoculated group	
		Diabetic (mg)	Regenerating (mg)
5	62.6 \pm 4.59	45.3 \pm 4.62	
20	159.0 \pm 10.57	71.5 \pm 7.26	170 \pm 40

8 to 10 mice of each group were used. Results are mean \pm SD ($P = 0.0003$).

Table 3. Changes in insulin content (μ g/g) of the pancreas in control and EMC-D virus inoculated diabetic and regenerating mice at different post-infection days

Days	Control group (mg)	EMC-D virus inoculated group	
		Diabetic (mg)	Regenerating (mg)
5	75.4 \pm 12.45	20.5 \pm 7.88	
20	77.8 \pm 5.71	25.1 \pm 6.44	33.2 \pm 8.22
45	88.7 \pm 3.75	15.3 \pm 7.87	78.6 \pm 7.65

8 to 10 mice were used in each group. Results are mean \pm SD ($P < 0.0001$).

cose > 450 mg/dl) which persisted for about 20–25 days post inoculation. A large number of mice in the experimental group died (20 out of 36) within 2 weeks post-inoculation. In the surviving mice, 75% (12 out of 16) of the mice exhibited signs of reversal of diabetes as revealed by decreasing levels of glucose in the urine and blood. The process of reversal of clinical diabetes began in the 3rd week post-inoculation and was completed after 6 weeks, as evidenced by the absence of glucose in urine and establishment of normoglycaemia (Table 1). In the remaining 25% of the population amongst the surviving mice in the experimental group, blood and urine glucose did not disappear, indicating continued diabetic status.

The reversal of clinical diabetes in the experimental group was further documented by the increase in total weight and insulin content of the pancreas (Tables 2 and 3). It is clear from Table 2 that on day 20 post-inoculation the weight of the pancreas in uninoculated control group (159.0 ± 10.57 mg) is comparable to that of regenerating mice (170 ± 4.0 mg) whereas it is significantly lower in diabetic mice (71.5 ± 7.26 mg). Similarly, there was a significant difference in the insulin content of the pancreas in the control and experimental group of mice on day 5 post-inoculation (Table 3) whereas on day 45 post-inoculation insulin content of pancreas was comparable in both the groups (Table 3), indicating regeneration of islets and documenting reversal of diabetes as revealed by clinical picture. The histopathological studies were carried out on pancreas of control and experimental group at day 5 and day 45 post-inoculation. Figure 1 shows normal histological architecture, islet morphology in control group, whereas Figure 2 shows loss of islet morphology, β -cell lysis and insulinitis in experimental group on day 5 post-inoculation. However, histological picture of experi-

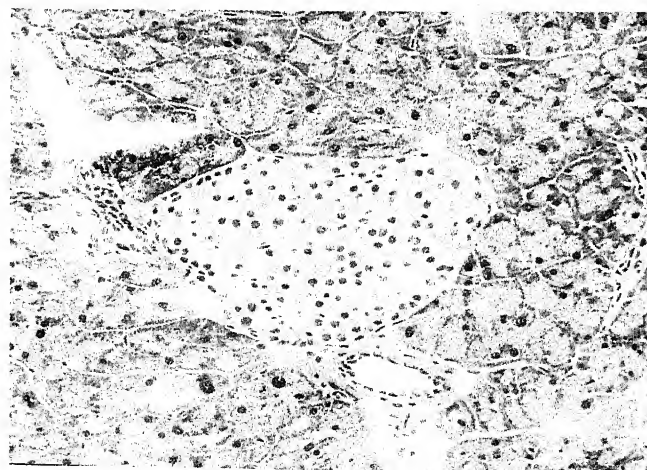


Figure 1. H&E stained section of pancreas from control mouse, showing normal islet surrounded by acinar cells ($\times 320$).

mental group pancreas on day 45 post-inoculation was comparable to that of control group of pancreas on day 45 except for smaller islets in the former (Figure 3).

It is shown in this study that a single injection of EMC-D virus can induce diabetes in SJL/J male mice within a week after virus inoculation by reducing the β -cell mass and insulin content of the pancreas. However, the remaining β -cell volume seems to be sufficient to regain normoglycaemia in at least 30% of the animals (12 out of 40). Taking into consideration the course and outcome of diabetes in the experimental group over a period of time it is seen that there are definite signs of reversal of diabetes as evidenced by the absence of glycosuria, achieving normoglycaemia and insulin content of the pancreas comparable to those of control group. The experimental data indicates reversion of EMC-D virus-induced diabetes. Such type of reversion from diabetic to nondiabetic status has not been reported previously in the case of EMC virus-induced diabetes. The reversal of diabetes obtained in the present study appears to be a case of spontaneous regeneration of the residual β -cell mass that was left intact after EMC-D virus infection. Thus, EMC-D virus-induced diabetes in SJL/J mice provides a new model to study the process of regeneration in pancreatic β -cells and reversal of diabetes. Such a type of spontaneous regeneration has been reported in streptozotocin (STZ)-induced diabetes in mice⁵, which provides another model to investigate mechanisms and promoting factors of such restorative processes. However, the present model differs from STZ-induced diabetic model⁵ in many respects. Firstly, our model involves adult mice as opposed to neonatal mice in STZ model. Secondly, the time of onset and the time of recovery are much earlier compared to the STZ model. The only limitation is the high mortality

rate in our model (55%) as opposed to STZ model. It is remarkable that the mice were able to recover in spite of severe hyperglycaemia, considering the fact that high blood glucose levels *per se* may have toxic effects on pancreatic β -cells⁸. This model also differs from other models of pancreatic regeneration such as partial pancreatectomy⁹ wherein there is 60–90% loss of whole pancreas, which results into regeneration of entire pancreatic tissue as opposed to selective loss and regeneration of only islet tissue as observed in the present study. The EMC-D induced diabetes model also differs from autoimmune models of diabetes such as biobreeding rat¹⁰ and nonobese diabetic mouse¹¹, wherein total destruction of β -cells occurs due to immune cytolytic process, thereby reducing the amount of residual β -cell mass and eventually reducing the chances of regeneration and recovery. It seems plausible to control the degree of damage of β -cells in the present model by reducing the dose of virus injected or by suppressing virus replication¹². Although the present study neither permits elucidation of mechanisms involved in the process of regeneration nor indicates origin of newly developed islets, the results obtained reflect an intrinsic limited capacity or potential of β -cell regeneration. Probably the recovery process might be caused by factors released from damaged β -cells, a mechanism observed in many other tissues after cell damage¹³. Since the number of functionally intact islets is of decisive importance for the development, course and outcome of diabetes, there is very little hope of recovery and regeneration of β -cells in insulin-dependent (Type 1) diabetes which is a consequence of progressive β -cell destruction^{14,15}. However, the recent report² on human pancreatic islet function at the onset of Type 1 diabetes clearly indicates the presence of insulin producing viable cells, suggesting a new

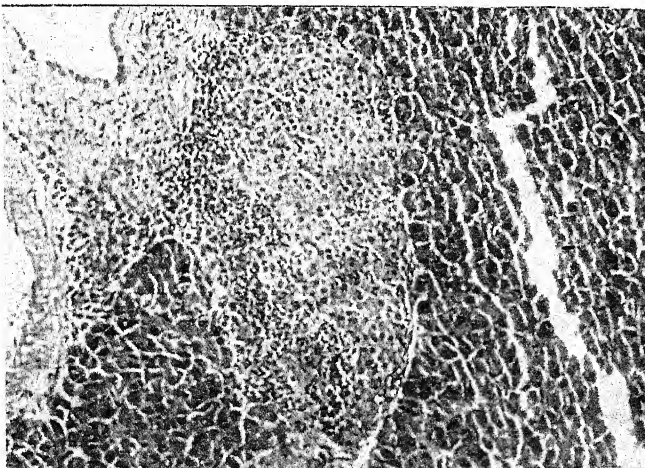


Figure 2. H&E stained section of mouse pancreas, 5 days after EMC-D virus infection showing extensive inflammatory infiltrate with mononuclear cells in the islets of Langerhans ($\times 320$).

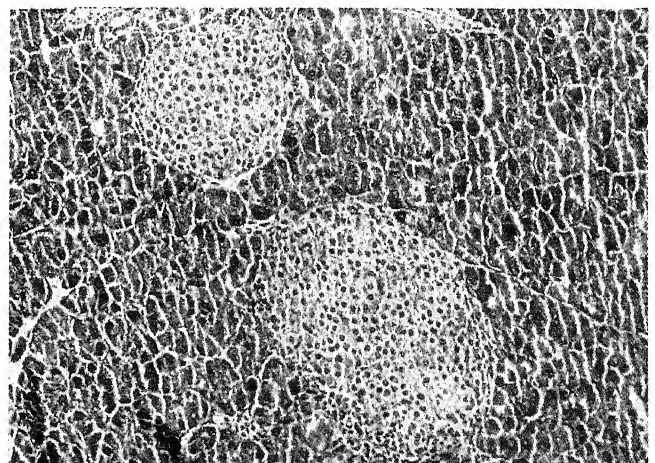


Figure 3. H&E stained section from regenerated mouse pancreas on day 45 exhibiting normal islet morphology comparable to that of the control group ($\times 320$).

dimension in the treatment of Type 1 diabetes. In this context, the results of the present study are of importance as they reflect on the intrinsic capability of residual islet cell mass to restore transiently after EMC-D induced decrease in β -cell number.

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Ascorbic acid counteracts the prooxidant effect of alloxan in erythrocytes *in vitro*

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Alloxan-induced experimental diabetes has been reported due to the production of toxic O_2^- and OH^\cdot radicals and H_2O_2 . Alloxan induced lipid peroxidation (LPO) in rat erythrocytes in the absence of ascorbic acid (AA). Superoxide dismutase (SOD) activity decreased while catalase (CAT) activity increased in erythrocytes with alloxan treatment without AA. Alloxan treatment in presence of AA showed no significant changes in LPO, SOD and CAT activities in erythrocytes, indicating neutralization of alloxan induced free radical production. Treatment with glucose in presence of AA showed no significant changes in LPO and in SOD and CAT activities in erythrocytes. Erythrocytes incubated with alloxan and glucose without AA showed increased LPO and decreased activity of SOD and CAT. However, LPO decreased and enzyme activities were comparable to control when treatment with alloxan and glucose was followed in presence of AA.

ALLOXAN is known to cause cytotoxicity to β -cells of

pancreas by producing highly reactive free radical species¹. The action of alloxan is inhibited by superoxide dismutase (SOD) and the enzyme has been shown to have therapeutic value in alloxan-induced diabetes². These and other studies showed the involvement of free radical species such as O_2^- and OH^\cdot radicals in the action of alloxan. In the present study, rat erythrocytes exposed to alloxan have been used as an *in vitro* model to show the action of alloxan. As the cyclic reaction involving alloxan and its reduced product dialuric acid spontaneously produce O_2^- and OH^\cdot radicals and H_2O_2 (refs 2, 3), it was intended to show whether alloxan causes changes in antioxidant enzymes in the erythrocytes. Further, whether addition of free radical scavengers such as ascorbic acid (AA) inhibits the action of alloxan. Ascorbic acid was used to investigate whether it protects erythrocytes from the prooxidant effect of alloxan. Glucose was added in physiological amounts to erythrocytes to compensate for any glucose loss during incubation and to observe the effects of alloxan in presence of glucose. In the present study, the action of alloxan *in vitro* and the protective action of AA on erythrocyte lipid peroxidation (LPO) and antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) were determined. The report indicates that alloxan induced LPO and changes the activities of SOD and CAT, and the action is mitigated by AA in the rat erythrocyte system.

Male Wistar rats weighing 150-180 g were housed in polypropylene cages under standard conditions with free access to drinking water and basal diet. The animals

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were sacrificed by decapitation and blood was collected in 2% citrated vials by heart puncture. Blood was centrifuged and erythrocytes were washed twice in 0.1 M phosphate buffered saline (PBS, 1:9, v/v) pH 7.4 and adjusted to 5% packed cell volume (PCV). The 5% PCV was incubated in 0.1 M PBS pH 7.4 along with glucose (5 mM), alloxan (100 mM) or both with (5 mM) or without AA and incubated at 37°C for 1 h in a well-oxygenated water bath. Alloxan, glucose and AA were prepared fresh in 0.1 M PBS pH 7.4 and added immediately to the mixture. In our earlier studies, the amount of alloxan used showed significantly increased LPO in erythrocytes incubated with alloxan^{4,5}. The erythrocytes were washed thrice in PBS and were used for the assays.

LPO was estimated as malonyldialdehyde (MDA) formed by thiobarbituric acid (TBA) reaction⁶. Two ml of 5% PCV was exposed to 10 mM H₂O₂ and 2 mM sodium azide in a total volume of 4 ml 0.1 M phosphate buffer pH 7.4. The mixture was incubated at 37°C for 1 h followed by the addition of 2 ml trichloroacetic acid (28%). The cell suspension was centrifuged at 1000 g for 5 min. Four ml of the supernatant was transferred to a boiling tube to which 1 ml TBA (1%) was added and the contents were boiled for 15 min, cooled immediately and the absorbance at 532 nm was recorded in a spectrophotometer. Blank sample was prepared without H₂O₂.

SOD and CAT were analysed in erythrocyte lysate prepared by the method of McCord and Fridovich⁷. Each enzyme assay was performed in duplicate in two-fold concentration range. The SOD activity was determined by the method of Marklund and Marklund⁸ by the ability of the enzyme to inhibit the autooxidation of pyrogallol. CAT was assayed by the measurement of the decomposition of H₂O₂ (ref. 9). Haemoglobin was estimated as described by Dacie and Lewis¹⁰. Protein content in the haemolysate was determined by the method of Lowry

*et al.*¹¹. The statistical analysis was done using Student's *t*-test and the probability level of less than 5% was considered significant.

Erythrocytes were exposed to glucose (5 mM), alloxan (100 mM) or both with (5 mM) or without the presence of AA (Table 1). Glucose in physiological amounts (5 mM) showed no significant change in LPO with or without AA compared to the respective control. Alloxan increased LPO in erythrocytes compared to its control in absence of AA. However, in presence of AA, alloxan treatment showed no significant change in LPO compared to control. Alloxan and glucose without AA stimulated an increase in LPO. However, such an increase in LPO was not observed in alloxan and glucose-treated erythrocytes in presence of AA. On the contrary, the presence of AA decreased LPO in erythrocytes induced by alloxan and glucose as compared to its control.

Erythrocytes in presence of glucose without AA showed increase in SOD and CAT activities (Table 2), however, glucose in presence of AA showed no significant increase in these enzymes as compared to respective controls. Alloxan treatment without AA decreased SOD activity while the CAT activity increased in erythrocytes as compared to respective controls. In presence of AA, alloxan showed no significant change in SOD and CAT activity as compared to control. Alloxan and glucose treatment without AA inhibited SOD and CAT activity in erythrocytes. However, such an inhibition in SOD and CAT activities by alloxan and glucose treatment was overcome in presence of AA.

Alloxan induced LPO and altered the reactive oxygen scavenger enzymes such as SOD and CAT, and the effect of alloxan was mitigated with AA in rat erythrocytes. The plasma AA at increased concentrations has been reported to be an effective scavenger of free

Table 1. Effect of various treatments on LPO in erythrocytes

Treatment	LPO (nmol MDA formed/h/g Hb)	
	- AA	+ AA
Control	277 ± 08	230 ± 10
Glucose	295 ± 16 NS	249 ± 09 NS
Alloxan	354 ± 11 <i>P</i> < 0.001	206 ± 15 NS
Alloxan + glucose	400 ± 16 <i>P</i> < 0.001	185 ± 22 <i>P</i> < 0.05

LPO was expressed as nmoles of MDA formed/h/g Hb. Values are mean ± SD of 6 animals. Erythrocytes were incubated in 0.1 M PBS pH 7.4 at 37°C for 1 h with glucose (5 mM), alloxan (100 mM) or both at the concentration shown, with (5 mM) or without ascorbic acid (AA). The values in treated groups were compared with respective controls. The value of LPO in erythrocytes without incubation (0 h) was 252 ± 10 nmoles of MDA formed/h/g Hb.

Table 2. Effect of various treatments on the antioxidant enzymes in erythrocytes

Treatment	SOD (units/mg protein)		CAT (units/mg protein)	
	- AA	+ AA	- AA	+ AA
Control	269 ± 11	275 ± 15	247 ± 06	269 ± 10
Glucose	349 ± 19 <i>P</i> < 0.05	299 ± 09 NS	362 ± 13 <i>P</i> < 0.001	315 ± 12 NS
Alloxan	200 ± 10 <i>P</i> < 0.05	257 ± 15 NS	329 ± 34 <i>P</i> < 0.001	298 ± 34 NS
Alloxan + glucose	133 ± 12 <i>P</i> < 0.001	298 ± 11 NS	168 ± 17 <i>P</i> < 0.001	264 ± 14 NS

SOD and CAT activities are expressed as units/mg protein and mmoles of H₂O₂ decomposed/min/mg protein, respectively. Values are mean ± SD of 6 animals. Erythrocytes were incubated in 0.1 M PBS pH 7.4 at 37°C for 1 h with glucose, alloxan or both with or without ascorbic acid (AA) at the concentrations shown in Table 1. The values were compared with respective controls. The value of SOD and CAT in erythrocytes without incubation (0 h) was 262 ± 9 units/mg protein and 267 ± 5 mmoles of H₂O₂ decomposed/min/mg protein, respectively.

radicals in aqueous phase, more effective than any other endogenous antioxidant¹². Alloxan induced LPO in rat erythrocytes, however, such an effect was not observed in presence of AA. Glucose in physiological amounts showed no significant change in erythrocyte LPO with or without AA. However, high amounts of glucose have been shown to produce toxic oxygen species in presence of transition metals¹³.

Alloxan with or without glucose increased oxidative reaction and generation of oxygen-free radicals. These effects of alloxan were observed in absence of AA. In presence of AA no significant increase in erythrocyte LPO was observed in alloxan-treated erythrocytes with or without glucose, suggesting the role of O_2^- anion in the induction of alloxan-induced LPO in erythrocytes. AA acts as scavenger of the free oxygen species, thus lowering LPO in erythrocytes treated with AA.

The increase in SOD and CAT activity due to glucose in absence of AA may be an attempt by erythrocytes to counteract any oxidative change. However, in presence of AA, no significant change was observed in erythrocyte SOD and CAT activities or in LPO, which indicate that free radical species are effectively neutralized by AA.

Alloxan due to the production of free radical species such as O_2^- inhibited SOD activity which may be one of the reasons for increased LPO in erythrocytes. The increase in CAT activity may be due to increased production of H_2O_2 in presence of alloxan. As the changes in SOD and CAT activity in alloxan-treated erythrocytes recover towards normal in presence of AA, it is suggested that these enzymes are modified in presence of alloxan and the action is mitigated by AA. Reactive oxygen species (ROS) not only induce LPO but also modify enzymes. The involvement of ROS in the modification of protein kinase C has been suggested to be an effective on/off signal mechanism to influence cellular events¹⁴. ROS has been shown to induce signalling including activation of protein kinase¹⁴, induce protein phosphorylation¹⁵ and also act as second messenger for the expression of genes involved in the immune response¹⁶. It is probable that ROS may modify reactive oxygen scavenger enzymes and in presence of AA may show dual activation-inactivation of these enzymes.

It is concluded that alloxan due to the production of free radical species increased LPO and altered antioxidant enzymes in erythrocytes. The treatment with glucose in physiological amounts showed no effect on erythrocyte LPO but increased SOD and CAT in glucose-treated erythrocytes in absence of AA. However, no significant changes were observed in LPO, SOD and CAT activities in erythrocytes treated with glucose in presence of AA. Alloxan and glucose treatment in absence of AA increased LPO and decreased SOD and CAT activities in erythrocytes. None of these changes was observed

in presence of AA. Thus, it may be one of the processes, how the cells respond to and mitigate the ill effects of reactive oxygen intermediates engendered in biological systems by diverse processes.

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Plant extracts: A non-chemical approach to control *Fusarium* diseases of mulberry

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Leaf extracts of *Azadirachta indica*, *Calotropis gigantea*, *Eucalyptus* sp., *Parthenium hysterophorous* and *Pongamia pinnata* were evaluated for their antifungal activity against *Fusarium pallidroseum* and *F. moniliforme* var. *intermedium* causing leaf blights, and *F. oxysporum* causing leaf spot diseases in mulberry (*Morus alba* L.). Leaf extract (1:5) of *P. pinnata* was highly fungitoxic to *F. pallidroseum* and *F. moniliforme* var. *intermedium* inhibiting their mycelial growth in plates by 78.2% and 84.3%, respectively; whereas *C. gigantea* and *A. indica* were most effective

against *F. oxysporum* inhibiting its mycelial growth by 78.5% and 73.2%, respectively. Under greenhouse conditions, aqueous leaf extract (25%) of *P. pinnata* reduced the incidence of leaf blights caused by *Fusarium pallidorozeum* and *F. moniliforme* var. *intermedium* by 63.6% and 67.1%, respectively; whereas in case of leaf spot caused by *F. oxysporum*, *C. gigantea* and *A. indica* both were effective in reducing the leaf spot incidence by 60.2% and 57.2%, respectively.

In mulberry, *Fusarium* diseases are the most destructive ones which are prevalent in all the southern states of the country where sericulture is generally practised. These diseases are observed in all the seasons with varying degree of disease incidence (3.0–19.5%) which reduce the leaf yield and its nutritive value, and make mulberry leaves unsuitable for silkworm feeding¹⁻³. Therefore, keeping in view the seriousness of *Fusarium* diseases in mulberry, and possible harmful effects of chemical fungicides on silkworm growth and development, there is a need to develop a non-chemical, eco-friendly method for the control of these diseases.

In the recent past, the angiospermic plants are proved to be a useful source of fungitoxic substances that are rather harmless compared to synthetic chemical fungicides which often impose undesirable side effects. Several plants have been reported to possess substances which are toxic to microbial pathogens and serve as protective barriers to infection⁴. The present investigation was, therefore, undertaken to test the efficacy of leaf extracts of *Azadirachta indica* A. Juss., *Calotropis gigantea* (L.) R. Br., *Eucalyptus* sp., *Parthenium hysterophorus* L. and *Pongamia pinnata* (L.) Pierre against *Fusarium* species, viz. *F. pallidorozeum* (Cooke) Sacc., *F. moniliforme* Sheldon var. *intermedium* Neish & Leggett and *F. oxysporum* Schlecht. causing leaf blights and leaf spot diseases in mulberry^{2,3}.

Twenty-five grams of fresh leaves of each plant species, viz. *Azadirachta indica*, *Calotropis gigantea*, *Eucalyptus* sp., *Parthenium hysterophorus* and *Pongamia pinnata* (washed well 2–3 times with tap water and once with distilled water) were ground in 100 ml of sterile distilled water. The macerate was squeezed through double-layered muslin cloth and centrifuged at 5,000 rpm for 20 min. The supernatant was filtered through Whatman No. 1 filter paper and then sterilized by passing through the Seitz filter (G5). The extract (25%) thus obtained was used for the *in vitro* experiments. For *in vivo* greenhouse experiments, the macerate obtained after squeezing through double-layered muslin cloth (25% aqueous leaf extract) was directly used for spraying plants.

For evaluation of antifungal effect of leaf extracts on mycelial growth of *Fusarium* species in plates, potato dextrose agar medium was amended with filter-sterilized fresh leaf extract (25%) to get dilution of 1:5, 1:10,

1:20 and 1:30 (extract:medium). The medium devoid of leaf extract served as control. The medium was poured into Petri plates (90×17 mm) at 15 ml/plate. The plates were inoculated centrally with a 4 mm mycelial disk (cut from the edge of 5-day-old actively growing colonies) of each species of *Fusarium*. Four replicates were maintained for each dilution and control. The whole set of experiment was incubated at 28±2°C and mean radial growth of fungal colony was recorded after a week. The fungitoxic efficacy of leaf extract was determined by comparing the radial growth in treatment (T) with the control (C). The inhibition percentage (I) was calculated by following the formula⁵: [I = (C – T/C) × 100].

Leaf extracts found effectively inhibiting the growth of *Fusarium* species on plates, were tested *in vivo* under greenhouse conditions. Extracts of *P. pinnata*, *C. gigantea* and *A. indica* were tested against leaf blights caused by *F. pallidorozeum* and *F. moniliforme* var. *intermedium*, whereas *C. gigantea* and *A. indica* were tested against leaf spot caused by *F. oxysporum*.

Mulberry (*Morus alba* L.) var. Kanva 2 plants, susceptible to *Fusarium* species tested, were raised from healthy cuttings and grown in earthen pots (40 cm) in a greenhouse at 28±2°C with relative humidity of 75%. Three sets of required number of potted plants (2-month-old) were inoculated separately with *F. pallidorozeum*, *F. moniliforme* var. *intermedium* and *F. oxysporum* by atomizing their freshly prepared uncontaminated spore suspensions (1×10⁶ conidia/ml) on to the leaves of plants. Inoculated plants were covered individually with a moist polythene bag for 24 h. After 72 h of inoculation, aqueous leaf extracts (25%) of *P. pinnata*, *C. gigantea* and *A. indica* were sprayed separately on to the leaves of plants inoculated with *F. pallidorozeum* and *F. moniliforme* var. *intermedium*; whereas extract of *C. gigantea* and *A. indica* were sprayed on to the leaves of plants inoculated with *F. oxysporum*. The extracts were sprayed twice at an interval of one week. Three replications of 5 plants each (total 15 plants) were maintained for each extract and *Fusarium* species, and the same number of plants from each *Fusarium*-inoculated set were sprayed with sterile distilled water which served as control. All the replications, including control, were arranged in a randomized block design. Disease intensity was recorded in treated and control plants after 30 days of second spray. From the observations, the per cent disease incidence (PDI) values (severity of disease) were calculated according to the FAO formula for plant disease assessment in different grades⁶.

$$PDI = \frac{\text{Sum of numerical values}}{\text{Total number of leaves observed} \times \text{Maximum grading (5)}} \times 100.$$

The sum of numerical values was obtained by mul-

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tipling the number of leaves observed in a particular grade with their respective grading as given below: 1=No infection; 2=up to 5% of leaf area affected; 3=6–25% of leaf area affected; 4=26–50% leaf area affected; and 5=51–100% leaf area affected.

From the PDI in control (PC) and treatment (PT), the per cent disease control (PDC) was calculated by following the formula: $[PDC = \{(PC - PT)/PC\} \times 100]$.

The per cent inhibition data (Table 1) were statistically analysed by subjecting them to ANOVA, whereas qualitative PDI data were analysed by following Mann-Whitney *U* test.

In order to confirm the results, each experiment was repeated three times.

Significant reduction ($p \geq 0.05$) in radial growth of *F. pallidoroseum*, *F. moniliforme* var. *intermedium* and *F. oxysporum* was observed with leaf extracts of *A. indica*, *C. gigantea*, *Eucalyptus* sp. and *P. pinnata*. However, extracts of *P. hysterophorus* stimulated the growth of all *Fusarium* species, even at a lower concentration of 1:30. The inhibition effect of leaf extracts was directly correlated with their concentration which increased with the concentration of extracts. Both the leaf blight causing *F. pallidoroseum* and *F. moniliforme* var. *intermedium*

showed similar trends of inhibition in their mycelial growth, however, *F. oxysporum* causing leaf spot disease differed from these two species in responding to leaf extracts.

Leaf extracts of *P. pinnata* was highly fungitoxic at all concentrations to *F. pallidoroseum* and *F. moniliforme* var. *intermedium* inhibiting their radial growth in plates by 63.1–84.3%; whereas *Eucalyptus* sp. was least effective to both *Fusarium* species, even at a high concentration of 1:5 as it showed only 30.5% and 22.0% growth inhibition, respectively. *C. gigantea* was moderately effective against both the leaf blights causing *Fusarium* species inhibiting 50.3–71.8% growth (Table 1 and Figure 1).

Leaf extracts of *C. gigantea* and *A. indica* were found to be most effective at all concentrations against *F. oxysporum* inhibiting 58.5–78.5% and 59.7–73.2% growth, respectively. *P. pinnata* extract, though highly fungitoxic to both the leaf blight causing *F. pallidoroseum* and *F. moniliforme* var. *intermedium*, exhibited least inhibition potential (0.8–12.2%) against leaf spot causing *F. oxysporum* (Table 1 and Figure 1).

Leaf extracts of *A. indica* and *P. pinnata* have earlier been reported inhibiting the mycelial growth of various

Table 1. Fungitoxic potential of leaf extracts against *Fusarium* species *in vitro*

Plant species	Conc. of extract	Inhibition/stimulation (+) percentage		
		<i>F. pallidoroseum</i>	<i>F. moniliforme</i> var. <i>intermedium</i>	<i>F. oxysporum</i>
<i>Azadirachta indica</i>	1:5	44.9	61.2	73.2
	1:10	38.5	58.2	69.4
	1:20	27.5	52.9	65.3
	1:30	24.7	50.9	59.7
		{0.12}	{0.04}	{0.03}
<i>Calotropis gigantea</i>	1:5	68.3	71.8	78.5
	1:10	62.6	67.6	73.4
	1:20	55.3	63.1	66.9
	1:30	50.3	58.5	58.5
		{0.07}	{0.03}	{0.03}
<i>Eucalyptus</i> sp.	1:5	30.5	22.0	21.1
	1:10	13.1	10.5	11.8
	1:20	6.3	5.5	4.4
	1:30	1.8	1.7	1.1
		{0.07}	{0.04}	{0.02}
<i>Pongamia pinnata</i>	1:5	78.2	84.3	12.2
	1:10	74.1	80.9	4.8
	1:20	68.8	72.8	2.0
	1:30	63.1	68.9	0.8
		{0.06}	{0.06}	{0.03}
<i>Parthenium hysterophorus</i>	1:5	+20.0	+23.9	+11.5
	1:10	+10.2	+15.3	+7.1
	1:20	+4.9	+6.3	+4.1
	1:30	+2.4	+3.8	+1.2
		{0.09}	{0.03}	{0.02}
C.D. $\{\geq 0.05\}$ between extracts.		0.03	0.15	0.01
C.D. $\{\geq 0.05\}$ between concentration of individual extract (in parentheses).				

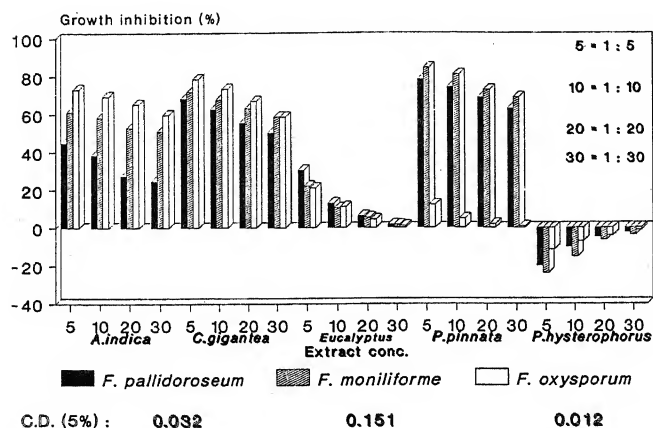


Figure 1. Fungitoxic potential of leaf extracts against *Fusarium* species *in vitro*.

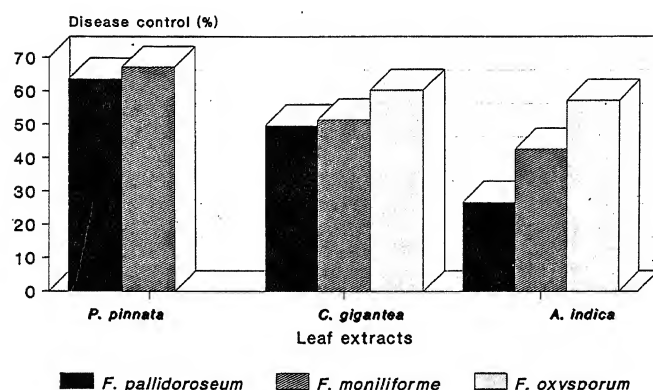


Figure 2. Efficacy of leaf extracts in controlling the *Fusarium* diseases of mulberry.

Fusarium species^{7,8}, which is in agreement with the present findings. Leaf extracts of *Eucalyptus* sp. and *C. gigantea* have also been known for their antifungal activity against various fungal pathogens^{9,10}. However, these extracts have not been studied against any species of *Fusarium*. The inhibitory effect of all these leaf extracts on mycelial growth might be attributed to the presence of antifungal ingredients in them^{8,9,11}. *P. hysterophorus* leaf extract, though reported for its antifungal properties against *F. oxysporum* and some other fungal pathogens^{12,13}, is found stimulatory to *Fusarium* species infecting mulberry. It may be due to the variations in response of different *Fusarium* species and formae speciales to the leaf extracts⁴.

In greenhouse experiment, leaf extracts of *P. pinnata*, *C. gigantea* and *A. indica* were proved to be fungicidal

as these extracts significantly controlled the leaf blights and leaf spot diseases caused by *Fusarium* species in mulberry. Aqueous extract (25%) obtained from the fresh leaves of *P. pinnata* showed better fungicidal potential by reducing the disease severity (PDI) of *F. pallidoroseum* and *F. moniliforme* var. *intermedium* leaf blights by 63.6% and 67.1%, respectively. In the case of leaf spot caused by *F. oxysporum*, leaf extracts of *C. gigantea* and *A. indica* were able to control the disease severity by 60.2% and 57.2%, respectively (Figure 2). These results are in agreement with the earlier observations¹⁰, particularly in case of *C. gigantea* and *A. indica* which have been reported to reduce the disease severity of leaf spot caused by *Cercospora moricola* in mulberry. However, contrary to the earlier report¹⁰, in the present study the leaf extract of *P. pinnata* is highly effective in controlling the leaf blights caused by *F. pallidoroseum* and *F. moniliforme* var. *intermedium* in mulberry. This is the first report on evaluation of leaf extracts for the control of *Fusarium* diseases of mulberry under realistic conditions in greenhouse.

All the leaf extracts tested were found to be fungicidal, but non-phytotoxic in nature. Thus, these extracts can be exploited for the control of *Fusarium* diseases of mulberry.

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Geochemical analysis of iron oxide/hydroxide coated and uncoated non-magnetic component of the bulk sample: Possible utility of the coated component in a stream sediment survey for gold

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The possible relationship between the secondary Fe-oxides/hydroxides and particulate gold in the surfacial environment is presented in this communication. Coated non-magnetic component separated from bulk, using isodynamic separator, shows much higher values for gold compared to the same of the uncoated. The increase in concentration is related to the possible affinity between the secondary oxides/hydroxides and particulate gold and the abundance of the two in the area. Possible affinity between these oxides/hydroxides and colloidal, fine/dust particles, etc. of gold may make the total coated component (coated magnetic + non-magnetic) useful to provide an additional data on adsorbed gold apart from particulate. Positive relationship between iron oxides/hydroxides and gold values suggests the utility of the total coated component in general and coated non-magnetic component in particular in stream sediment survey for gold.

THERE are many reports in the literature about the presence of Fe-oxide/hydroxide coating in secondary environment, particularly in the zone of weathering and its relation to gold¹⁻⁵. It is also observed that the lateritic gold enrichment can be associated with the formation of colloidal gold and its interaction with the iron-oxide-rich medium. Adsorption of colloidal gold on synthetic goethite and hematite was measured experimentally at 25°C and it was observed that the affinity rate depends more on the composition of the medium. Inert electrolytes, if present, are expected to enhance the effect⁶. This information provides the impetus to study the relationship between the iron oxide/hydroxide coating and the particulate gold.

The shortcomings in the sample-processing scheme to separate iron oxide/hydroxide-coated particulate gold from heavy mineral concentrate⁷ and its implications on gold exploration are discussed earlier by the author⁸. The utility of another component, i.e. the total iron oxide/hydroxide-coated component in general and coated non-magnetic component in particular of the bulk sample in gold exploration is reported here.

Stream sediment samples collected earlier during an orientation survey for gold from an area known for Au and Cu mineralization near Bhiwapur, Nagpur district, Maharashtra⁷ were used in this study. Details about

geology of the area, sampling, etc. are provided elsewhere⁷. About 1 kg of partly iron oxide/hydroxide-coated -30+50 mesh size (-0.59+0.297 mm) bulk sample was run in a isodynamic separator at 1.2 Å (25° forward/15° side slope) to separate magnetic and non-magnetic components⁷. Magnetic component in this case contains iron oxide/hydroxide-coated magnetic and non-magnetic minerals plus uncoated magnetic minerals. So, both coated magnetic and non-magnetic minerals form the total coated component present in the sample. Since we mainly deal here with the coated non-magnetic component, the total coated component was treated with dilute HCl (to remove coating) before further separating the earlier coated non-magnetic minerals⁷. The non-magnetic component thus separated at two stages firstly, from bulk and secondly from the total coated component, was analysed separately for gold.

Fifty grams of the first and 20 g of the second stage non-magnetic component were separately ground approximately close to -120 mesh size. It was then transferred into a beaker and treated with 50-60 ml acid mixture (hydrobromic acid + few drops of bromine solution)^{7,9}. The sample was then washed and transferred into a 100 ml volumetric flask containing 10 ml iso-butyl methyl ketone (MIBK). After shaking the solution for few minutes, the organic layer was analysed for gold using AAS (Perkin-Elmer, 2380) at Department of Geology, University of Delhi. The data thus generated are presented in Table 1.

The secondary oxides/hydroxides of Fe, Mn, etc. known to be active in the surfacial environment, not only coat the grains⁷ of different sizes but also facilitate accumulation of ionic, colloidal⁶ dust or fine particles^{1,2}, etc. of different minerals or elements through absorption and adsorption processes. In the present case, about 4-22%

Table 1. Gold data (in ppm) on iron oxide/hydroxide coated and uncoated non-magnetic component and approximate percentage of coated non-magnetic and total coated component in the bulk sample

Sample number	A	B	C	D
1	0.1	0.6	10	20
2	0.1	0.4	11	22
3	0.1	0.5	7	14
4	< 0.1	0.2	6	12
5	0.1	0.3	7	14
6	0.1	0.2	6	12
7	< 0.1	0.3	7	14
8	< 0.1	0.2	4	8
9	< 0.1	0.1	6	12
10	< 0.1	0.1	3	6
11	< 0.1	< 0.1	3	6
12	0.1	< 0.1	2	4
13	< 0.1	0.1	3	6

A, Uncoated non-magnetic of the bulk sample.

B, Coated non-magnetic of the bulk sample.

C, Approximate percentage of coated non-magnetic component.

D, Approximate percentage of total coated component (i.e. coated magnetic + non-magnetic).

(assuming to be twice the amount of coated non-magnetic grains/minerals (Table 1)) of the grains ($-30+50$ mesh size) are estimated to be coated by these oxides. Thus it is important to understand the pattern of coating either selective or random. Since these oxides are also known to coat the gold grains⁷, it is also important to understand the relationship between them in terms of geochemical survey.

Data (Table 1) generated on coated non-magnetic component ($<0.01-0.6$ ppm) compared to that of the uncoated ($<0.01-0.10$ ppm) component show much higher values for gold. These enhanced values not only suggest the possible affinity between these oxides/hydroxides and gold but also the relationship of particulate gold with the intensity of these coatings. Since about 2–11% of the bulk sample comprises iron oxide/hydroxide-coated non-magnetic grains, the values recorded for gold in the non-magnetic component either low (of first stage) or high (of second stage) are expected to be influenced by these percentages. It is well reflected in the gold values of the coated non-magnetic component where sample no. 1, coating being approximately 10%, shows 0.6 ppm and sample no. 9, with 6% shows only 0.1 ppm. This relationship, though not exactly linear is also seen in Figure 1.

The data not only confirm the earlier report on gold nuggets recovered from such similar component⁷ but also indicate the possible utility of such coated non-magnetic component in a stream sediment survey for gold.

The above observation together with the experimental data on colloidal gold⁶ probably indicate the possible affinity of these oxides not only to particulate but also to colloidal, dust/fine particles, etc. of gold in the secondary environment. Though the proportion of gold (different forms) that is going to be enriched in the presence of these oxides is not clear, their role in influencing particulate gold values (Table 1) and colloidal gold⁶ is better understood. Thus, coatings become useful to provide information on both particulate and adsorbed gold. In the case of particulate gold, only the coated non-magnetic component, i.e. 2–11% (present case) is utilized. Whereas in the case of adsorbed gold the total coated component about 4–22% (i.e. coated magnetic + non-magnetic) is utilized.

Hence, the presence of iron oxide coating on the one hand affects the separation of particulate gold in the case of HMC⁷ and on the other may provide useful information on colloidal, dust/fine particles, etc. of gold, apart from particulate (Table 1) in the case of total coated component of the bulk sample.

Further, it is suggested that there is a need for a suitable sample-processing scheme, where the secondary iron oxides/hydroxides are conspicuous, to recover both

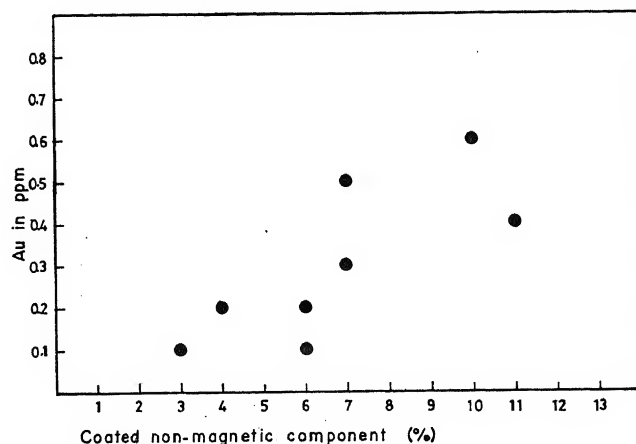


Figure 1. Positive relationship between the percentage of coated non-magnetic component vs gold concentration.

absorbed and adsorbed gold apart from particulate from bulk sample.

To conclude, iron oxide/hydroxide-coated non-magnetic component yields higher values for gold (particulate) compared to the uncoated component. Possible affinity among Fe-oxide/hydroxide and colloidal⁶, dust/fine particles, etc. of gold makes the total coated component important for adsorbed apart from particulate gold. Since a positive relationship is observed between the intensity of coating and gold values (of particulate), a similar trend may be expected also in the case of adsorbed gold. Coated component like heavy mineral concentrate seem to have some application in geochemical survey for gold, particularly in areas where iron oxide coating is quite conspicuous.

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Chitinozoa and melanosclerite from the Lower Paleozoic sequence of the Tethyan Garhwal Himalaya – A note on their identification and distinction

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Sediments of the Shiala and Yong Limestone formations belonging to the marine Lower Paleozoic sequence in the Tethyan Garhwal Himalaya have yielded a rich assemblage of acritarchs. In addition to prolific occurrence of acritarchs, these sediments have also yielded rare to infrequent chitinozoa and melanosclerite. It is pointed out that the distinction between these two distinct palynological entities is rather difficult through the conventional microscopic examination. The present paper highlights the subtle and minor differences in the morphologies of the two which could be seen only through SEM. Thus, a thorough rechecking and revision of earlier records is necessary for correct identification.

The present study reveals the occurrence of rare presence of chitinozoa and melanosclerite along with prolific acritarchs¹ from the Shiala and Yong Limestone formations. These formations of the Tethyan Garhwal Himalaya are well exposed in and around the village Sumna (30°40'N, 80°50'E) of the Chamoli district of the Garhwal Division, Uttar Pradesh. The lithostratigraphic framework at Sumna has already been described^{2,3} (Table 1).

The Garbyang Formation grades into the Shiala Formation without any lithological change². Four biostratigraphic zones have been established based on macrofauna and Middle to Upper Ordovician age has been assigned to the Shiala Formation^{2,3}. However, Middle Ordovician age is assigned to the Shiala Formation based on conodont species⁴. But, Caradocian to Wenlockian age is assigned to the Shiala Formation based on the acritarch assemblages recorded¹. The Yong Limestone is biohermal in nature and is recognized as a separate lithostratigraphic unit, named as the Yong Limestone Formation which conformably overlies the Shiala Formation. An Upper Ordovician to Lower Silurian age has been assigned to the Yong Limestone^{2,3,5}. However, Sinha *et al.*¹ have dated the Yong Limestone Formation to be of Late Silurian age (Ludlovian) based on acritarch assemblage.

The method of preparation of strato-litho-petrographic column and the traverse direction for sample collection along the Sumna-Rimkhim section has been described elsewhere¹.

The samples collected from the Shiala and the Yong Limestone formations are clastics and non-clastics which include mainly fine-grained calcareous quartz arenite, siltyshale and wackstone (bioclast).

From each sample, 50 g of pea-sized rock material was dissolved first in hydrochloric acid and then in hydrofluoric acid and finally in nitric acid. The macerated and decomposed material was washed four times with distilled water by centrifuging. Material thus obtained was treated with heavy liquid using KI, CdI₂ and ZnI. The permanent slide was prepared by adding 2 drops of polyvinyl alcohol into the residue. All the slides and samples are deposited at the Department of Earth Sciences, University of Roorkee for future reference.

Chitinozoans are a group of acid-resistant microfossils whose biological affinities are yet to be ascertained⁶⁻⁸. It is believed that they represent the eggs or egg capsules of a soft-bodied metazoan^{9,10}. They occur singly or in colonies⁷, containing twelve or more individual vesicles⁶. Few chitinozoans show polymorphism phenomenon⁷. Eisenack¹¹ first recorded chitinozoans in Germany in 1931. Chitinozoans have been described from almost all the continents, with the single exception of Antarctica¹². They are exclusively marine planktic microfossils found in sedimentary rocks ranging in age from Ordovician to Devonian⁸. They have been reported from Mississippian rocks also¹³. They occur in Paleozoic shale and limestones; and are found to be rare in coarse grained limestone and sandstone due to the winnowing action of prevailing current¹⁴. They are generally flask-shaped¹⁴ and range in size from 60 to 2,000 microns¹⁵ but the majority of species have a size range between 100 and 300 microns⁶. Due to their rapid evolution, wide distribution and short ranging species of chitinozoa permit a detailed biozonation of Palaeozoic strata^{8,15}. It has been shown that the detailed chitinozoan biozonation is possible in sediments that have undergone greenschist facies metamorphism¹⁶. The first global chitinozoa biozonation for the Silurian has been proposed based on short ranging, well-defined and easily identifiable index

Table 1. Lithostratigraphic framework of the Tethyan Garhwal Himalaya (Sinha, 1989)

Time unit	Lithounit	Generalized lithology
Silurian	Yong Limestone Formation	Green nodular limestone
Ordovician	Shiala Formation	Sandstone, quartzite, limestone, alternate bands of sandstone and shale, alternate bands of greenish shale and limestone
	Garbyang Formation	Green needle shale with occasional bands of limestone, sandstone and shale



Figure 1 a-t. Palynological slide photographs. Figures a, b, d, e, g, i-t are either chitinozoans or melanosclerites. a, sample no. R-44(3), coordinate 110 × 56.6, L = 60 µm. b, sample no. R-43(1), coordinate 101.5 × 43.6, L = 75 µm. d, sample no. R-38(3), coordinate 105.7 × 55.3, L = 54 µm. e, sample no. R-39(1), coordinate 110 × 50, L = 70 µm. g, sample no. R-37(1), coordinate 108.5 × 27.3, L = 105 µm. i, sample no. R-50(2), coordinate 112.2 × 49.3, L = 240 µm. j, sample no. R-35(2), coordinate 99.6 × 49, L = 60 µm. k, sample no. R-37(1), coordinate 101.1 × 38.9, L = 120 µm. l, sample no. R-37(1), coordinate 98.9 × 40.6, L = 120 µm. m, sample no. R-37(1), coordinate 97.5 × 44.5, L = 110 µm. n, sample no. R-41, coordinate 99.5 × 17, L = 50 µm (thin section). o, sample no. R-37(2), coordinate 97.6 × 50.7, L = 62 µm. p, sample no. R-43(2), coordinate 105 × 45.4, L = 54 µm. q, sample no. R-40(1), coordinate 105.9 × 46, L = 82 µm. r, sample no. R-43(2), coordinate 97 × 43.5, L = 112 µm. s, sample no. R-44(2), coordinate 104.1 × 39.6, L = 50 µm. t, sample no. R-40(1), coordinate 107.6 × 59.5, L = 54 µm. Chitinozoans: c, *Desmochitina* sp., sample no. R-40(1), coordinate 95.1 × 35.8, L = 44 µm. f, *Ancyrochitina* sp., sample no. R-20(1), coordinate 93.9 × 51, L = 48 µm. h, *Desmochitina* sp., sample no. R-40(1), coordinate 91.5 × 61.5, L = 66 µm.

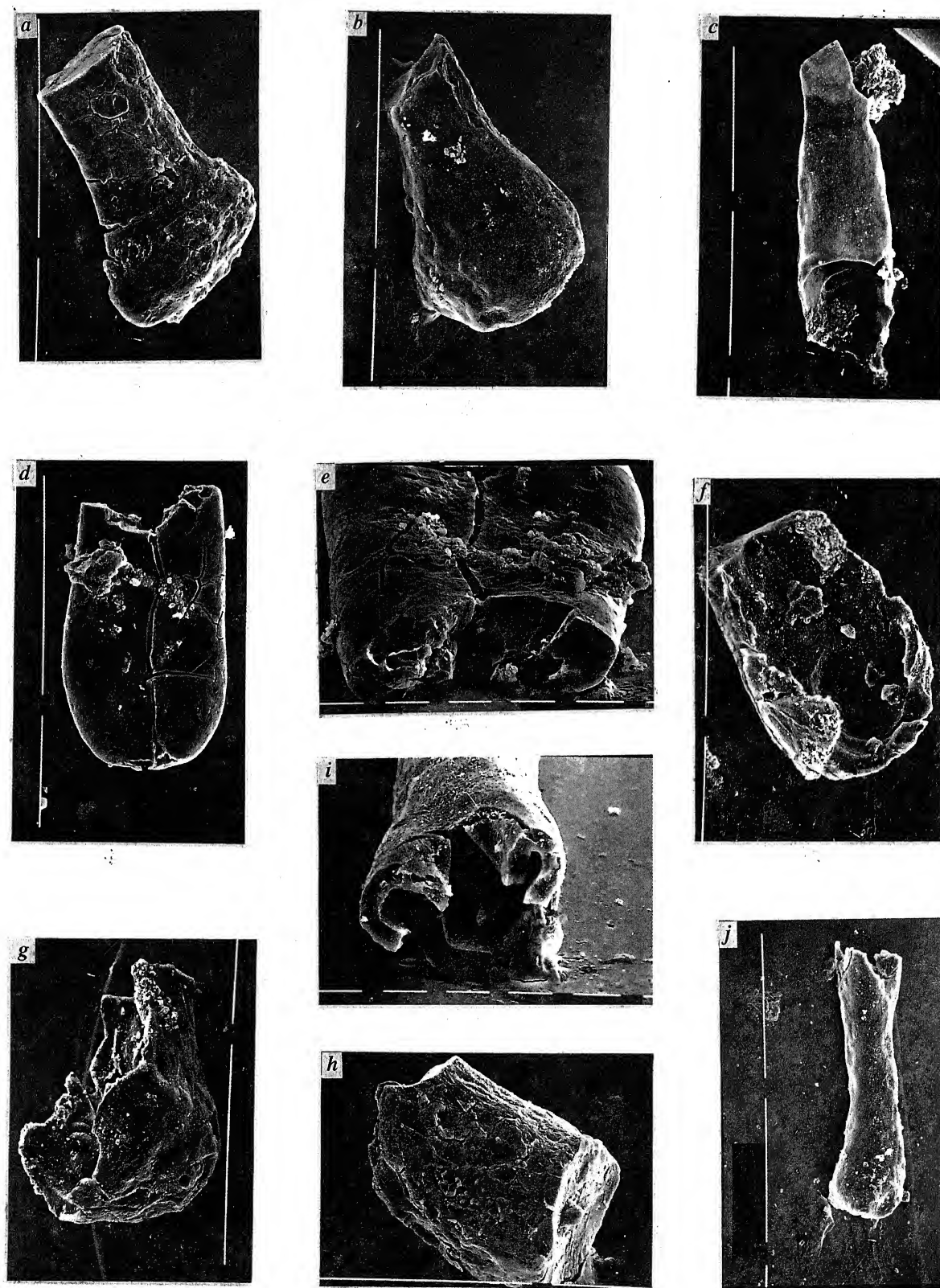


Figure 2 a-j. SEM photographs. *a, b*, Melanosclerites: *a*, sample no. R-43 ($\times 750$), *b*, sample no. R-41 ($\times 750$) (not hollow); *c*, aff. *Conochitina* sp. (broken), sample no. R-43 ($\times 500$); *d, e*: melanosclerite, *d*, sample no. R-43 ($\times 750$); *e*, showing thickness of the wall of figure *d* ($\times 1500$); *f, g*: looks like a *Bursachitina* sp. but is likely a melanosclerite, *f*, sample no. R- 41 ($\times 750$), *g*, tilted view ($\times 750$); *h*, fragment of melanosclerite, sample no. R-42 ($\times 750$); *i, j*: melanosclerite, *i*, thick wall ($\times 1500$), *j*, sample no. R- 41 ($\times 500$).

Table 2.

Features	Chitinozoans	Figure no.	Melanosclerites	Figure no.
Thickness of wall	Thin walled*	2 c	Thick walled*	2 d, e, i, j
Nature of vesicles	Hollow vesicles	2 c, f, g	Generally not hollow	2 a and b
Nature of central canal	—	—	Very small central canal	2 h

Light microscopic studies shown in Figure 1 a, b, d, e, g, i-t (Palynological slide) could either be chitinozoans or melanosclerites.

*Thickness of wall of melanosclerites is relatively more than the thickness of wall of chitinozoans in general.

species⁸. The chitinozoans are found to be most diverse and abundant in the subtidal deposits¹⁷.

The palaeogeographical studies of chitinozoan distribution are scanty¹⁸. However, the chitinozoan provincialism is less pronounced in Silurian than in the Ordovician on generic level^{18,19}.

The chitinozoa described from the Shiala and the Yong Limestone formations are black and very glossy under normal light (Figure 1). The black colour of fossils may be due to progressive increase of the carbon ratio, i.e. overmaturity²⁰ caused by eometamorphism²¹. Cramer *et al.*²¹ suggested that the black colour of chitinozoa is possibly due to the temperature exposure of about 200°C.

The term 'Melanosclerite' was first used by Eisenack²² to identify the problematic microfossils ranging in age from Ordovician to Middle Devonian (Eifelian age)²². Melanosclerites are exclusively marine rod-shaped microfossils with pseudochitinous wall composition²³, i.e. not very close to true chitin but similar to the composition of the shells of the Recent *Thecamoeba Gromia oviformis*²⁴. They have not been widely studied and are considered to have some affinity with hydrozoans²³. An algal origin for melanosclerites is also suggested²⁵. They are strongly facies controlled and restricted in sediments formed under open ocean conditions²⁶. However, Cashman²³ found a similarity in ecology and latitudinal occurrence between the modern cubomedusa, *Carybdea alata* and melanosclerite *Melanostylus coronifer*. This could be helpful in palaeoenvironmental and palaeogeographical reconstructions. More study on this problematic microfossil is required for better understanding on the phylogenetic history and their distribution through Phanerozoic. This would help in biostratigraphy and palaeoenvironmental interpretation²³.

The melanosclerites and chitinozoans recovered from the Shiala and the Yong Limestone formations of the Tethyan Garhwal Himalaya are poorly preserved. The chitinozoans recovered by the earlier workers are from the Vindhyan Supergroup (Sone Valley)²⁷; Pin dolomite, Spiti²⁸; Yong Limestone, Garhwal⁵, Spiti²⁹ and Satpuli, Garhwal³⁰. However, the published literature has no record of chitinozoans from the Shiala Formation. In the present study, the generic identification of some of

the recovered chitinozoans from Shiala and Yong Limestone formations has been done, which are long ranging in age. They are *Ancyrochitina* sp., *Desmochitina* sp., *Conochitina* sp. (Figures 1 and 2). The generic and specific identification of melanosclerites was not attempted during the present study.

Owing to a very close similarity in size and nature of silhouette of chitinozoans and melanosclerites, there exists every possibility of erroneous identification. It was found that many previously described and illustrated chitinozoan taxa proved to be unworkable when light microscope studies were replaced by routine SEM investigation¹². Thus, a form identified through a microscope as a chitinozoan may in fact be a melanosclerite. Hence, it may be pointed out that the earlier reports, records and illustrations published particularly from Indian subcontinent may require reexamination, checking, correction and revision through SEM.

In view of this information, the present material was carefully handled and examined before sending for publication.

The principal distinguishing features (which can be seen only under SEM) between the two distinct palynological entities are tabulated in Table 2.

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Gas-charged sediments in shallow waters off Redi along the central west coast of India

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This study reports the occurrence of gas-charged sediments in the nearshore areas of the west coast of India. High resolution shallow seismic reflection profiles on the nearshore area along central west coast of India, at water depths of 11-18 m are characterized by anomalous seismic subbottom signatures in the form of acoustic turbidity which extends from the underlying reflector and reach within 4-5 m of the seafloor. Fine grained sediments apparently contain appreciable quantities of interstitial gas bubbles which are responsible for the anomalous seismic sig-

natures. This could be due to shallow hydrocarbon gases, mostly methane, which might have been derived due to the biogenic degradation of organic matter accumulated under palaeo-estuarine conditions. These gas-charged sediments pose a potential hazard for the offshore engineering constructions. Hence, the detection of these sediments forms an essential part of any offshore site investigation programmes.

The western continental shelf is widest off Cambay (345 km) and it narrows down (60 km) off Quilon. It is characterized by gently dipping seafloor, with an average gradient of 1:400-1:3000, and the shelf break occurs at 80 to 140 m water depths. The inner shelf up to 50-60 m water depths is marked by even topography and is carpeted by silts and clays while middle and the outer shelf show uneven to rough topography with a thin layer of calcareous sands.

Recent developments in marine geophysical techniques have made it easier to identify gases in shallow marine sediments. Based on shallow seismic, side scan sonar and echogram results, gas-charged sediments have been reported from most of the world's continental shelves¹⁻⁵. The addition of gaseous phase compounds to the otherwise fluid saturated seafloor sediments significantly alters the acoustic properties of the seafloor^{6,7}. These effects produce readily identifiable anomalies in high resolution subbottom reflection profiles of the shallow seafloor⁸⁻¹⁰. Though the occurrence of shallow gas-charged sediments is documented on the western continental margin of India using seismic signatures¹¹⁻¹⁴, little or no similar information is available so far regarding these in nearshore areas. Here we report the occurrence of gas-charged sediments in the nearshore areas of the west coast of India.

The study area lies in the shallow waters off Redi,

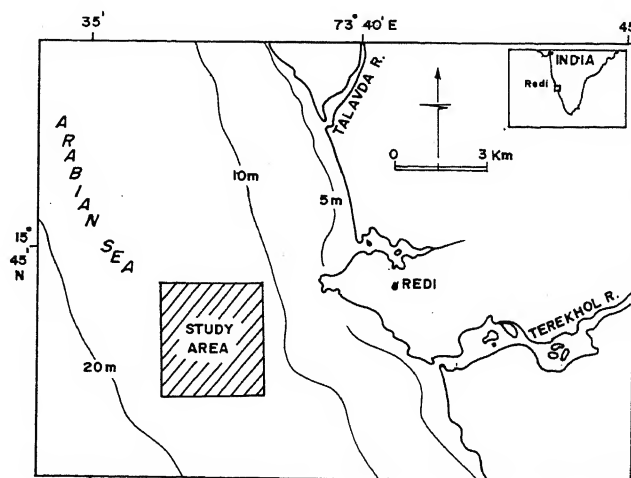


Figure 1. Map showing the survey area off Redi. Inset shows the location of Redi along the west coast of India.

about 400 km south of Cambay (Figure 1). Our data base comprises echosounding (Atlas Deso-10, 210 and 30 kHz), side scan sonar (EG&G, Mark IB, dual channel recorder 259-3 and tow fish 272) and high resolution shallow seismic profiling (300 joules and 400-5000 Hz). The survey tracks are approximately spaced 25 m apart in north-south and east-west grid fashion (Figure 2). The positions of the tracks were fixed by Mini Ranger MRS III system and a total of 500 line kms were covered.

In the study area, water depth ranges from 11 to 18 m and is dominated by gently westward sloping seafloor and it lies off the mouths of the rivers Talavda and Terekhol. The area is essentially featureless with the seafloor formed by silty clays. Shallow seismic records indicate that acoustically transparent clays with an assumed velocity of 1.5 km/sec range in thickness from 10 to 15 m cover the seabed. These clays are underlain by sandy reflectors. These acoustically transparent clays are marked by a sudden appearance of extensive patches of shallow gas-charged sediments in the form of 'acoustic turbidity', at places resembling inverted U-like features, where the reflections are chaotic caused by the scattering of the acoustic energy (Figure 3 a, b, c). The velocity in the gas-charged sediments is generally decreased depending upon the degree to which gas bubbles in

sediments act as a resonant system and the relation of acoustic frequency to the bubble-sediment resonance frequency¹⁵.

Acoustic turbidity is the term generally used to refer to those parts of the seismic studies where subbottom details are lost due to the effects of gas bubbles within the sediment pore spaces⁴. These acoustic turbid masses reach within 4-5 m of the seafloor. The sides of the turbid zones are nearly vertical. Their top edges are either flat or rounded. The records are similar to some of the published records of gas-charged sediments reported earlier^{1,11,13,14} in the outer shelf. The area covered by the present study is roughly 12 km² and it is estimated that 10-15% of the area is characterized by gas-charged sediments.

The anomalous features are confined only to the shallow seismic records and are not seen either in the corresponding echograms or in the sonographs. This reveals that the anomalous features due to gas are localized within the sediments but not surfacing out of the seabed, though upward migration of gases by either molecular diffusion or bubble formation from the sediments into the overlying waters could be possible¹⁶.

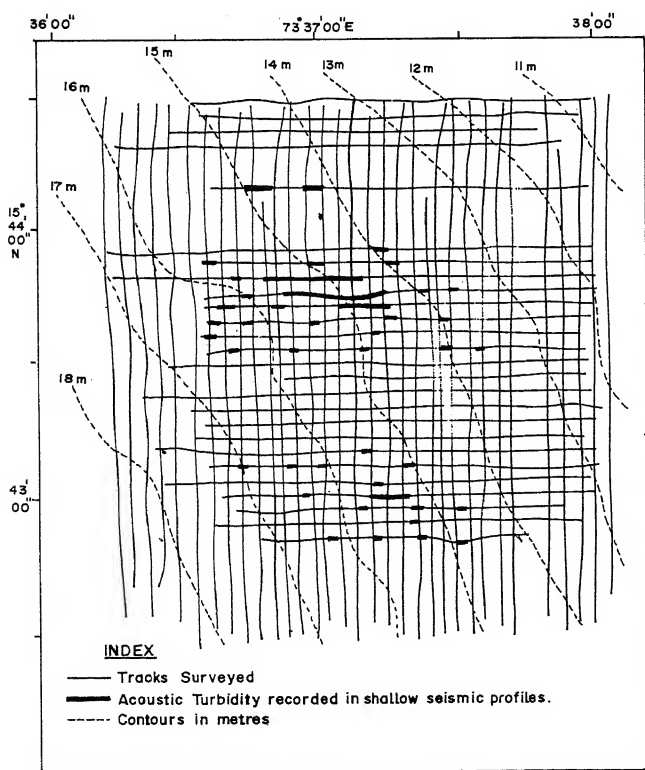


Figure 2. Survey tracks along which bathymetric, side scan sonar and shallow subbottom seismic profiling were carried out.

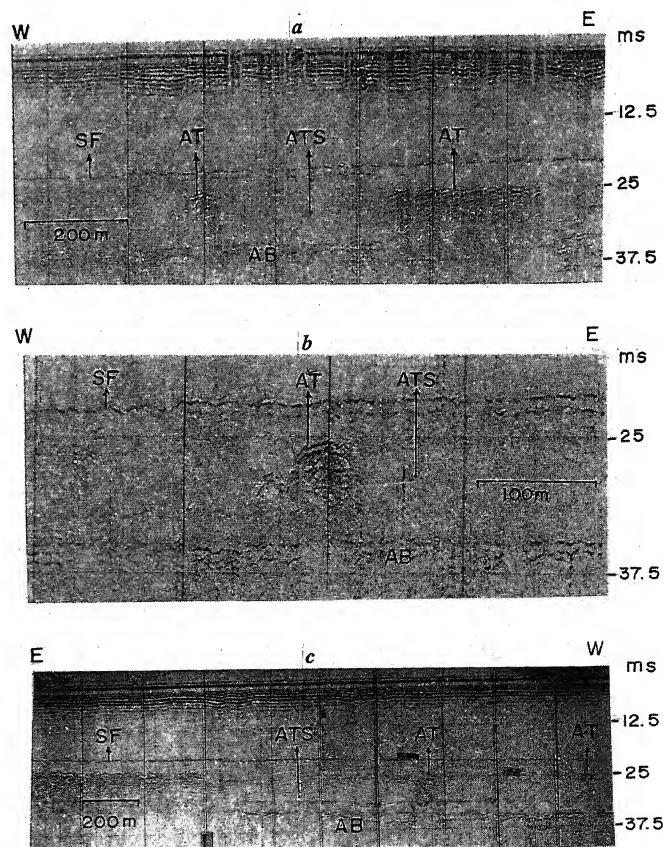


Figure 3 a-c. Seismic records showing examples of acoustic turbidity on high resolution seismic reflection profiles (300 J and 400-5000 Hz).

Shallow hydrocarbon gas, mostly methane has been reported from the western continental margin of India^{11-14,17} and from Amazon submarine deltaic regions¹⁸ elsewhere. According to Kaplan¹⁹, gases in the marine sediments could be from four different sources, viz. atmospheric, biogenic degradation of organic matter, upward diffusion by thermocatalytic cracking of more complex compounds and submarine volcanic or geothermal processes. Siddiquie *et al.*¹¹ indicated two probable possibilities for the gas on the western shelf, i.e. biodegradation of organic matter or thermocatalytic cracking of more complex compounds. Similarly Karisiddaiah *et al.*¹³ believed that these gases could be from biogenic sources or geothermal processes or both. Karisiddaiah and Veerayya¹⁴ emphasized the role of vegetation as the source for the accumulation of methane gas. Paropkari *et al.*²⁰ reported high organic carbon content in the western shelf of India. Mascarenhas *et al.*²¹ reported peat deposits intercalated in sediments from the inner shelf.

It is known that bacterial methane formation is common in more rapidly accumulating marine sediments with higher organic matter contents once the dissolved sulphate has been utilized²². Obviously, the primary requirement for the existence of such gases is the availability of sufficient organic matter below the seafloor and appropriate time for generation of sufficient gas molecules and enough permeability of the sediments required for gas to collect as bubbles²³.

The proximity of the study area to the mouths of the Talavda and Terekhol rivers, gives the high possibility that the offshore sediments may be sufficiently organic-rich to generate sufficient quantities of biogenic gas and to produce the observed seismic signatures. High rate of sedimentation at the river mouths which results in rapid burial of organic matter must have also triggered the gas formation. Since the occurrence of methane gas in the outer shelf sediments is reported earlier¹¹⁻¹⁴, this gas is also believed to be methane and of bacterial origin. As mentioned earlier, similar occurrences of shallow gas formations from deltaic sediments and from nearshore sediment containing organic-rich layer have been reported from the world's continental shelves²⁴⁻²⁸.

Methane is a powerful greenhouse gas^{29,30} which plays an important role in atmospheric chemistry by influencing both the ozone concentration and the infrared radioactive flux³¹. Similarly, the various effects of gas on the geotechnical behaviour of fine grained soils create the potential hazard for offshore construction and development. When the gas gets trapped and accumulates under an impermeable layer, the resulting gas pressure goes up to such a level that a blowout can occur during drilling. Similarly, gas escaping naturally to the surface in extreme cases causes the collapse of structures due to undermining of the foundation⁴. Hence, the gas

detection studies now form an essential part of any marine site investigation programmes.

Undoubtedly, there could be similar shallow sedimentary methane reservoirs in the Indian shelves associated with major buried palaeo-deltas of the rivers, buried fluvial systems, infilled palaeo-river channels, nearshore bays, estuaries, etc. More detailed and systematic studies of these areas, incorporating high resolution seismic subbottom profiling, will certainly unravel the reservoirs of natural gas accumulation.

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MEETINGS/SYMPOSIA/SEMINARS

Workshop on Fundamentals of Photosciences: Application to Basic Research

Date: 7-12 October 1996

Place: Calcutta

Topics include: Spectroscopic processes, viz. absorption, emission, NMR, ESR, etc. and their instrumentation; photodynamics using lasers and conventional flash lamps; photosynthesis, photomedicine, application of chiro-optical methods, synthetic photochemistry, etc.

Contact: Dr P. K. Bhattacharya
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National Conference on Moriculture: Physiological, Biochemical and Molecular Aspects of Stress Tolerance in Mulberry

Date: Second half of October

Place: Tiruchirapalli

Topics include: Molecular aspects of stress tolerance; *In vitro* induction of stress tolerance; Physiology and biochemistry of mulberry in stressed environment; Environment of rhizosphere microflora (VAMF, ecto, microbes of extreme environment) in conferment of stress tolerance.

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National Seminar on Neotectonic Movements and their Geo-environmental Impacts (NETMOGEI)

Date: 27-29 November 1996

Place: Mysore

Themes include; Tectonic framework and deformational behaviour of Peninsular shield (Deep continental crust, Deccan volcanic province, BGC, Coastal boundaries); Geomorphological, hydrological and sedimentological signatures of tectonically formed/active zones; Geophysical signatures of crustal blocks (Conductivity, velocity models, deep reflection, magnetotellurics, and allied aspects); Petrological, structural and geochemical signatures of tectonic zones (Pressure, temperature, fluid flux history, dating, emission of helium and radon, and allied aspects); Modelling of tectonic processes, boundaries, earthquake expectancy, simulation strategies and constraints; Hazard zone mapping, distribution of epicentres, palaeoseismic sites and drainage reversals; Remotesensing applications to neotectonics and structural fabrics; Impact assessments of neotectonic activities and damage; Modernization and strengthening of monitoring networks.

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The inventor of the jet engine

An obituary of Sir Frank Whittle

There was a certain poignancy to the news that Sir Frank Whittle, who could, if any one could, be called the inventor of the jet engine, had passed away on 10 August 1996, at the age of 89. This was because so few were even aware that this remarkable man was still now, alive; this age has no patience with modesty. I came to hear of his death from a small obituary in the *Deccan Herald*. And there was an unintended irony even here: the obituary of this living legend was directly below a column listing the winners of one of our numerous scientific awards. Whittle, an introvert of sorts, had a hard time getting any recognition at all, while now, especially in our country, recognition usually goes to those adept at self-advertisement who shout the loudest or who have someone to shout for them. It is not easy to be heard in the noisy market place; such are our times.

Frank Whittle was born in Coventry, Warwickshire, on June 1, 1907, to parents of apparently modest means. At the age of 16 he entered the Royal Air Force as an apprentice, studied at the Royal Air Force College, the Central Flying School and the RAF School of Aeronautical Engineering, Cranwell, before being posted to a fighter squadron in 1928. Besides teaching flying, Whittle served as a test pilot at the Marine Aircraft Experimental Establishment, Felixstowe, during the years 1931–32. This was followed by further studies at the RAF engineering school and at the University of Cambridge. While his basic ideas on

the jet engine date back to 1928, when he was a young cadet in the RAF, his confidence in the correctness of these ideas must have grown with his increased technical knowledge acquired during his post-graduate studies. Thus he was well prepared to put his ideas into practice when shortly before the war, in 1937, he was attached to Power Jets Ltd., to develop gas turbines for the jet propulsion of aircraft.

Without an historical perspective it would be impossible, especially at a time when jet travel is common place, to properly assess the immensity of Whittle's achievement. He was among the first, if not the first, to conceive of a well-defined engine based on jet propulsion. He was the first to show, using general thermodynamic arguments, that the engine was technically feasible. With a small staff of a couple of draughtsmen and a few other assorted assistants he then designed the component parts of the engine, i.e. the compressor, the combustor, the turbine, etc; he then had these built and then did the testing himself at considerable personal risk to himself and his small staff. Against almost impossible odds he had the prototype engine built to be test flown on a specially built Gloster E28/39 airframe. That first test flight on May 15, 1941, was successful. So the man conceived the idea, worked out the details, supervised the manufacture and had the prototype test flown, with the assistance (after 1937) of a staff of less than 10 people and a total cost (after 1937) of

a few thousand pounds! A human and engineering saga that would thrill even the most jaded.

The rest of the story, however, is depressing. Once the idea was proved successful, the forces that be decided that the invention was too important to have its further development be entrusted to as small a man as Frank Whittle. He was made an adviser to the Ministry of Supply in 1946, retired from the RAF with rank of Air Commodore in 1948, and in the same year knighted and bought off with a government award of 100,000 pounds, tax free! But no more involvement with the love of his life. Considering the current size of the jet engine business, the sum awarded appears ridiculously small in these days of intellectual property rights and the Bill Gateses. A story repeated many times since the days of Archimedes of Syracuse. It appears that his intense disappointment, at having failed to gain control over his invention, led to a nervous breakdown. He emigrated to the United States soon after, retiring in 1979 as an adjunct professor at the US Naval Academy.

I first came to know of Whittle's inspiring story from a superb biography that I accidentally found at the Indian Institute of Science library in the early seventies. Neither during my engineering college days nor during my days as a graduate student had I heard of his remarkable achievements; nor did I ever come across him holding forth at a conference or hear of him at one of these. This quiet genius had done his work, single handed, and then simply disappeared from the scene making no claims for himself or his achievements. To those of us who prefer our heroes in the heroic mould of a previous age, when greatness was measured by what you had actually done, Whittle belongs, along with a few other loners of his generation like Bethune and Turing, in the first rank of these.

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Sir Frank Whittle

Annual Review of Microbiology 1994. Nicholas Ornston, Albert Belows and E. Peter Greenberg, eds. Annual Reviews Inc., 4139 El Camino Way, Palo Alto, California, USA. Vol. 48. Price: \$139. 834 pp.

While it is late to review the 1994 issue of this series at this time, several articles in the issue drew my attention. The first and foremost was the opening article 'A Charmed Life' by Boris Magasanik, senior lecturer at MIT, Cambridge, MA, USA. I was influenced by one of Boris' chalk board presentations during an in-house symposium in the honour of H. G. Khorana at his retirement a couple of years ago. Hence, it was with great pleasure that I went through this article. Reading through his biography was a reminder of how careers of scientists of his time depended on the events of the World War II and, in his case he even had to serve in the US army in England and France for nearly four years. In spite of this, his scientific career has been very illustrious. Immediately after completion of the army services, he joined Chargaff's group at the Columbia University for his PhD to work on inositol metabolism and completed his degree in 1948. Just then Chargaff had begun his studies on the composition of nucleic acids, and Boris continued for one more year to work on chemical and enzymatic degradation of RNA before joining as faculty at the Harvard Medical School in 1949. Boris' alertness to the recent trends in scientific research helped him utilize the bacterial genetics to the best advantage in isolating a number of auxotrophic mutants of *Klebsiella aerogenes*, *Salmonella typhimurium* and *B. subtilis*. These mutants proved to be instrumental in his studies on inositol metabolism, purine and histidine biosynthesis and some earlier concepts on catabolite repression, both at the Harvard Medical School and later at MIT when he moved there in 1960. Subsequently these studies paved a way for him to study the elegant mechanism of regulation of expression of glutamine synthetase (*glnA*) by the regulatory proteins NRI (NtrC) and NRII (NtrB) in bacteria.

This volume of the Annual Reviews is also important in that it contains a good collection of articles on the unique biology of trypanosomes, a parasitic protozoan responsible for a number of dis-

eases in humans and domestic animals. Pays, Vanhamme and Berberof review the mechanisms of differential and stage-specific developmental gene expression of variant surface glycoproteins (VSGs) and procyclins responsible for antigenic variation in the host. These organisms are unique in that modulation of promoter activity does not play an important role in gene expression. Instead, gene rearrangements, change in chromatin structure, and various aspects of posttranscriptional and translational controls are mainly responsible for regulation of gene expression. Of particular interest to me was that the presence of a novel nucleotide, β -D-glucosylhydroxymethyluracil in the telomeric regions of the bloodstream forms correlated with the inactivation of expression sites possibly as a result of alterations in the chromatin structure. In bloodstream form, glycolysis is the major pathway used by the parasite for energy generation. Sommer and Wang have contributed an article that provides details on targeting of proteins to glycosomes (organelles that compartmentalize glycolytic enzymes). Detailed knowledge of targeting signals should prove useful in developing therapeutic reagents which can be targeted to glycosomes to inhibit glycolysis. *Trypanosoma brucei brucei* is infectious to domestic animals but it is rendered noninfectious in humans because of the presence of the cytolytic activity against this strain in normal human serum. However, this cytolytic activity does not restrict the disease-causing strains, *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*. High density lipoprotein (HDL) was characterized to be responsible for the lytic activity. Hajduc, Hager and Esko have reviewed the high density lipoprotein-mediated killing of the trypanosomes. Biochemical characterization suggests apolipoprotein A1 to be the active ingredient in this killing process. While the exact mechanism for the lytic activity of HDL/apolipoprotein A1 is not clear, various models that lead to internalization of HDL/apolipoprotein A1 and subsequent lysis of the parasite have been discussed. In any case, these observations could be very important in development of HDL-based therapeutic reagents against trypanosomes. Another aspect of trypanosome biochemistry on characterization of *trans*-sialidase (TS) has been described by Schenkman,

Eichinger, Pereira and Nussezeiw. This enzyme catalyses transfer of sialic acids from host glycoconjugates to acceptor molecules of the parasite plasma membrane. Interestingly, *T. cruzi* (causative agent for Chaga's disease) expresses this enzyme both in the insect vector and the human host whereas the African trypanosomes (causative agents of sleeping sickness) express this enzyme only when in the insect vectors. This enzyme has been characterized and its gene has also been cloned. This enzyme is important in that it performs glycosyl transfers without using a nucleoside diphosphate substrate. Yet another article by Zilberstein and Shapiro provides details on the role of the environmental factors such as pH and temperature on stage-specific gene regulation of a related genus, *Leishmania* responsible for various human diseases.

Feagin reviews on extrachromosomal DNAs of apicomplexan (sporozoa) parasites. Well-known examples of these protozoan parasites are the *Plasmodium* species. Other parasites that cause diseases in humans include *Toxoplasma gondii* and *Cryptosporidium* species. Several others such as *Babesia*, *Theileria* and *Eimeria* cause diseases of domestic animals. What is most interesting of the mitochondrial DNA in these organisms is the presence of the ribosomal RNA (rRNA) sequences in discontinuous regions resulting in fragmentation of the rRNAs. These fragments correspond to only the highly conserved regions of the rRNAs. Variable region sequences of the rRNAs have been dispensed with. This 'gene shrinking' experiment that the nature has performed should be extremely useful for structure function analysis of the rRNA.

Further, Gerhart, Wagner and Simons have contributed a review on the regulation of expression by antisense RNA in prokaryotes. The article is very informative and a good starting point to learn 'everything you wanted know but were afraid to ask' about how the copy number of various plasmids is regulated by antisense RNA. The chapter also describes the role of antisense RNA in regulation of bacteriophage and in Tn10 (Tet^R) gene expression. Conveniently, general aspects of both the later topics have been reviewed by Campbell and, Hillen and Berens, respectively in the same issue. The book also contains a chapter by Loewen and Hengge-Aronis

on regulation of prokaryotic gene expression by σ in the stationary phase of the growth.

In retrospect, the review on prion proteins by Prusiner turned out to be a remarkable and ahead of its time contribution. The article journeys through the chronological developments of how from total denial, it became an established fact that the protein molecule alone is the infectious agent which causes scrapie of sheep, bovine spongiform encephalopathy (BSE) of cattle and Creutzfeldt-Jacob disease (CJD), fatal familial insomnia (FFI), etc. of humans. It is striking that a conformational change of some α -helical regions of the prion protein to β -pleated sheets converts a normal protein into an infectious agent. With the recent outbreak of BSE in UK, this article will definitely be a thirst quencher to many curious biologists.

The book also includes reviews on many other infectious organisms such as mycoplasmas and their role as cofactors during HIV infection. Several articles have been devoted on the aspects of microbial physiology as well as on industrial production of economically important products. Overall, the editors – L. Nicholas Ornston, Albert Belows and E. Peter Greenberg – have put together a good collection of articles worthy of appreciation. Most certainly, one cannot go wrong in acquiring this book for a personal collection.

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Dyke Swarms of Peninsular India. T. C. Devaraju ed. Geological Society of India, Bangalore. Memoir 33. 1995. 451 pp. Price: Rs 500, \$ 50.

Dyke swarms constitute an important feature of the earth's crust. These intrusive bodies represent major thermal and deformational events of the earth's long history and thus provide useful constraints to monitor the evolutionary processes leading to the development of crust and mantle. Like other shields of the world the Indian shield has also received voluminous amount of magmatic

material in the form of dykes and other shallow level intrusions at distinct time intervals. However, unlike other geological features, the dyke rocks of Indian Peninsula have not received due attention from geoscientists. Even details on their geological occurrence and distribution in time and space are lacking in the literature. The Geological Society of India, which has the reputation of bringing out monographs on such topics, has fulfilled a long-felt need of researchers working particularly in the field of petrology, geochemistry and tectonics which can provide up-to-date information on dyke rocks of peninsular India. Although a considerable amount of data in terms of geological occurrence, and major, trace and rare earth elements and also the isotopes are now available on their eruptive counterparts, the dyke rocks have generally been neglected. This monograph is an attempt to fill this gap for the benefit of researchers engaged in determination of chronology of geological events in peninsular India. The book covers not only some of the more significant features of dyke swarms of peninsular India, but also deals with some more important general aspects of dyke rocks.

The first three papers in the monograph are concerned with the review of research work done on general aspects of dyke rocks. These papers provide a good insight into some important features of dyke suites like flow and crystallization (by I. M. Platten), palaeomagnetism (by M. E. Evans) and clouding of feldspars (by H. C. Halls and Boaxing Zhang).

The next two papers deal with some general aspects of Precambrian dyke swarms of South Indian shield. On the basis of occurrence, distribution and age data, N. G. K. Murthy has identified five episodes of dyke activity ranging in age from 2240 m.y. to 75 m.y. A review of palaeomagnetic data on some dyke rocks of Indian shield is presented and discussed by V. Damodara Reddy *et al.*

The next three papers present case histories of some important Precambrian dyke swarms of southern Peninsula. Amitabha Sarkar and A. K. Mallik have discussed the isotopic and geochemical data to draw interpretations regarding age and petrogenesis of dykes of Kolar Gold Field. Two episodes of Proterozoic dyke activity are identified which the authors have tried to correlate with general dyke-forming events of Indian shield. T. Radhakrishna and J. Mathew have

presented a paper on mafic dyke swarm occurring in high-grade terrain of south India. Isotopic and geochemical data of advance nature are adequately used to interpret some important conclusions regarding evolution of Indian lithosphere. A detailed account of geochemical data on mineral phases of a dyke from Karnataka is presented by T. C. Devaraju and others for an understanding of differentiation processes.

A. B. Roy and others who have been authors/co-authors of some of the most informative papers on the Aravalli belt in the past, have contributed a paper on the dyke rocks of this region. The paper reveals seven dyke-forming events in Aravalli history. The occurrence of felsic dykes is of special significance. Another paper on dyke rocks of western Indian shield is by N. Kochhar and co-workers. These authors have presented the petrology and the geochemistry of acid and mafic dykes of Trans-Aravalli region associated with Jalor magmatic activity. This activity is considered to have been manifested in response to an abortive attempt of rifting in the Trans-Aravalli part of Indian lithosphere at about 750 m.y. ago.

The mafic dyke swarms of central Indian shield are dealt with in the paper by H. M. Ramachandra *et al.* The paper describes the occurrence of many dyke swarms in the region and provides details of Bhanupratappur–Keskul mafic dyke swarms. On the basis of field occurrence and geochemistry, the authors have discussed their petrogenesis and tectonic setting and find a close affinity of these dyke swarms with the famous Labrador and Scourie dyke swarms.

An interesting case history pertaining to dyke swarms of southern Karnataka is given by T. C. Devaraju and others in two papers. The authors have contributed 66 pages on these dyke swarms covering field characteristics, petrography, mineral chemistry, whole rock geochemistry and isotopic compositions. The data are adequately used to evaluate the relative significance of petrogenetic processes in the magmatic evolution of these dyke swarms and to interpret their tectonic setting.

J. Mallikarjuna Rao and others have discussed the field occurrence and K–Ar and Ar–Ar ages and geochemistry of dyke swarms related to Cuddapah basin and draw interpretations regarding their petrogenesis and tectonics. These

authors find an episodicity in Cuddapah magmatism between a period from 1850 to 1000 m.y., with a peak at about 1400–1100 m.y. However in another paper Y. J. Bhaskara Rao *et al.* have discussed their Rb–Sr age data on Pulivendla sill of Cuddapah basin. These authors have placed an 1800 m.y. age limit of a single major episode of mantle magmatism and Cuddapah basin formation.

The rest of the papers deal with various aspects of dykes and dyke swarms occurring along the western coast of India and Narmada rift, related with Deccan volcanism. K. B. Powar and S. V. Vadetwar discuss the geological occurrence, mineral chemistry and whole rock chemistry of dykes and plugs occurring in Revas–Murud area of Konkan coastal belt. The compositional variation from basaltic dolerite to gabbro-diorite is considered to be a consequence of fractional crystallization of a common magma. The status of these intrusives as feeders is rejected. S. F. Sethna and M. Mousavi present the major and trace element data on dykes and associated intrusions of Deccan basalts occurring on the western coast. These authors find influence of differentiation and crustal contamination on the chemistry of these rocks. Their derivation is interpreted in terms of batch partial melting of a heterogeneous mantle source. Another paper on the dyke rocks of western coast is by A. G. Desai and A. A. A. Viegas. The authors have identified four generations of dyke swarms which are related with tectonic evolution of western Indian continental margin.

A detailed account of field characteristics, structural setting, petrography and geochemistry of alkaline–tholeiite dykes associated with Amba Dongar and Phenai Mata complexes of lower Narmada valley is presented by L. G. Gwalani and others. R. V. Karanth and D. A. Sant have contributed on dyke swarms of southern Saurashtra. They have identified seven generations of dyke swarms which were emplaced along deep seated fractures related to Narmada rift zone.

Although the monograph covers almost all parts of Indian shield where current research is being carried out, there are certain important areas which are not represented. A glaring omission is Proterozoic dyke swarms of eastern Indian shield, i.e. the Newer Dolerites. It would have been better if a paper had

been included on these dyke rocks. The editor has done an admirable job by giving a summary in the beginning of the book with specific mention of the importance of dyke swarms in the studies of evolutionary history of crust and mantle, which is a real help to the reader. However, the absence of author and subject indices may be felt by the readers. The printing and get-up are good, size of letters is large enough but in some of the papers the size of diagrams particularly those of maps showing distribution of dykes is small.

Overall, the monograph is a welcome addition to the literature on Indian shield. The book, which contains a wealth of basic scientific data on dyke rocks of Indian shield, is worth possessing by any geoscientist.

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Annual Review of Astronomy and Astrophysics 1994. Vol. 32. Geoffrey Burbidge, ed. Annual Reviews Inc., 4139 El Camino Way, Palo Alto, California, USA. Price: USA \$ 60, elsewhere \$ 65. 662 pp.

This volume of *Annual Review of Astronomy and Astrophysics* contains fourteen articles covering a wide range of subjects from The Goldilocks problem: climatic evolution and long-term habitability of terrestrial planets to Baryonic dark matter and Recycled pulsars. All the topics are of great current interest and quite a few of them are relatively new areas of research. For example, the article by Brown and Gilliland on 'Astroseismology' is a first in the Annual Review series, although Deubner and Gough wrote on 'Helioseismology' more than a decade ago. During the last decade astroseismology has come of age and is providing a new way of looking at the interiors of stars. Brown and Gilliland's review is a very timely account of the progress in the field and of the promise it holds for the future. Similarly, the article 'Anisotropies in the Cosmic Microwave Background' could not have been written

much earlier as the observational breakthrough came only in 1992 with the first detection of large angular scale anisotropies of cosmological origin in CMB.

Much of the exciting progress in astronomy in the recent times has come about through advancements in technology, launch of new missions in space, commissioning of large ground-based optical telescopes (of aperture greater than 6 m), etc. Much of what has been reviewed in the volume carries the stamp of these developments. Infrared observations have revealed the existence of large quantities of very hot gas in clusters of galaxies. Refinements in radioastronomical techniques have led to accurate determination of isotopic and elemental abundances in the interstellar medium. Space missions have breathed new life into the studies of cosmic dusty plasmas. All of these subjects have been reviewed by respective experts in these fields. The references at the end of each of these articles with hardly any citations earlier than 1980 reveal how current the work reported on is.

While stellar physics absorbed the attention of the astronomers in the immediate post-war decades of this century, it is galaxies and their evolution which command the maximum interest in the current period. We now have the capability of routinely observing stars in other galaxies and more importantly we can observe them in the ultraviolet, the optical and the infrared. Comprehensive studies of stellar populations in other galaxies are now possible. Global properties of galaxies as a function of their morphological types are better understood. Deeper questions regarding the formation and evolution of stellar populations in galaxies can be addressed. Massive stars provide most of the light of the galaxies and the cooler stars most of the mass. The massive stellar population is best studied in the ultraviolet, the cooler red population in the near infrared. Panchromatic studies are therefore of great importance. The Hubble Space Telescope (HST) is providing spectacular pictures of external galaxies, starburst nuclei, quasars and their environment, distant clusters, etc. For the distant galaxies the light from the massive stars is redshifted and infrared observations (e.g. K band photometry) reveal many of the details that are usually seen in the optical observations of the nearby galaxies.

BOOK REVIEWS

Reviews by Roberts and Haynes (The Hubble sequence) and Maeder and Conti (Massive star populations in nearby galaxies) touch upon many of these crucial aspects of extragalactic studies.

Bigger than the galaxies and their clusters is the Universe itself and its origin and evolution occupy the attention of a very large number of theorists and observers. Today Physical Cosmology is an observational/experimental science in which definite physical models are used to interpret and predict the results of measurements. Results from the COBE satellite, from the HST key project to determine extragalactic distances to greater accuracy and from high resolution spectroscopy of faint distant galaxies with the Keck telescope have opened up hitherto inaccessible ways of investigating the cosmological problem. Many of the well-established notions of the Standard Big Bang Cosmology are being severely tested, the cosmological ques-

tions are being further sharpened. What is the age of the Universe? Is the Universe going to expand forever? How much of the matter in the Universe is in the dark form? How did galaxies form? When did they form? These and many other related questions are exercising the minds of the cosmologist, the astronomer and the particle physicist and a great debate is on. This volume 32 of the Annual Review brings us a flavour of these issues through the articles of Carr, White, Scott and Silk, Dekel and Fabian. There is a lot to ponder over and a lot to marvel at.

I shall be remiss if I do not mention the Prefatory Chapter of this volume. Margaret Burbidge is the author of it and her contribution 'Watcher of the skies' is a delight to read. Burbidge has been one of the distinguished astronomers of our times and the account of her life as a professional astronomer is of great common interest. As she says, the ARAA

Editorial Board invited her to write an account that would acquaint an observational astronomer today 'with what it was like to use optical telescopes before TV, 2-dimensional photon counting devices and computers'. She has succeeded in her job admirably. We now have in ARAA Prefatory Chapters accounts by three of the famous foursome B²FH.

Astronomy and astrophysics are a rapidly progressing field. Many of the results reviewed in this volume will either be improved upon or superseded by new developments in future. By monitoring this progress annually through superbly written articles, ARAA is doing a signal service to the astronomical community.

D. C. V. MALLIK

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MEETINGS/SYMPOSIA/SEMINARS

National Seminar on Biotechnology: New Trends and Prospects

Date: 25-27 November 1996

Place: Hardwar

Themes include: Microbial preparations and plant products; Education and research in biotechnology- Indian priorities; Microbial technology and renewable energy; Biotechnology for agriculture; Biotechnology in health care and food biotechnology.

Contact: Prof. D. K. Maheshwari
Convenor
Unit in Microbiology, Department of Botany
Gurukul Kangri University
Hardwar 249 404
Phone: 427871

National Symposium on Environment III: 1996. Eco-friendly and Biorational Pollution Management in Soil, Water and Air

Date: 10-12 December 1996

Place: Pune

The above seminar will be held in Hindi at National Chemical Laboratory, Pune. Themes: a) Pesticides: Alternatives - plant products, IPM, biopesticides etc.; b) Industrial effluents; c) Early warning systems: Role of biomonitoring; d) Miscellaneous.

Contact: Dr R. N. Sharma
Convenor, Head, Entomology
National Chemical Laboratory
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CURRENT SCIENCE

A fortnightly journal of research

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Current Science is a multidisciplinary journal and therefore research and review papers of general significance that are written clearly and well organized will be given preference. All papers will be first assessed by a Reviewing Editor. Papers found unsuitable in terms of the overall requirements of the journal will be returned to the authors. The others will be sent for detailed review. *Both solicited and unsolicited material will be reviewed.* Authors of these papers will be notified of acceptance, rejection, or need for revision of the paper. Returned papers cannot be resubmitted. Illustrations and other material to be reproduced from other publications must be properly credited; it is the authors' responsibility to obtain permission for reproduction (copies of letters of permission should be sent).

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Review articles (not exceeding 5000 words) are expected to survey and discuss recent developments in a field. They should be well focused and organized, and avoid a general, 'textbook' style.

Research articles (not exceeding 4000 words) should report research results of fairly major significance. They should include an abstract not exceeding 100 words, introductory paragraph(s), and brief subheads.

Research communications (not exceeding 2000 words) should contain important findings that are novel and of fairly broad interest. They should include a brief abstract and an introductory paragraph. Text should not be broken up under subheads.

Correspondence includes letters, not exceeding 500 words, that are of general interest to scientists. All letters cannot be published.

Scientific correspondence contains technical comments, including those on articles or communications published in *Current Science* within the previous six months. Letters may be reviewed and edited.

Research news articles are intended to inform nonspecialists about recently published advances or important findings discussed at a meeting. Authors should also send a copy of the paper(s) on which the article is based. Meeting reports should avoid merely listing brief accounts of topics discussed, and must convey to readers the significance of an important advance.

Research accounts articles are intended to be personalized reviews of research from the authors' own laboratory, based on a body of published work. The articles must provide appropriate background to the area in a concise introduction, which should also serve to place the author's work in proper perspective. Articles will normally

not exceed 8 to 10 printed pages.

Opinion articles present views on issues related to science and scientific activity. **Commentary** articles should contain expository notes on issues related to science and scientific activity.

Book reviews. Unsolicited reviews will also be considered. Reviews that merely 'list' brief descriptions of the contents cannot be published. Reviews should have 'context' and convey some information about the subject of the book.

Historical commentary and notes inform readers about interesting aspects of personalities or institutions of science or about watershed events in the history/development of science; most are expected to relate to India. Illustrations are welcome. Brief items will also be considered.

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Text. All papers should have a brief introduction. The text should be intelligible to readers in different disciplines and technical terms should be defined. Tables and figures should be referred to in numerical order. All **symbols** and **abbreviations** must be defined, and used only when absolutely necessary. Superscripts and subscripts and ambiguous characters should be clearly indicated. **Units of measure** should be metric or, preferably, SI. Methods should, as far as possible, be described briefly in appropriate table and figure legends.

Figures. In the case of line drawings one set of originals (without any lettering) is sufficient, with two copies containing lettering. In the case of photographs good prints are required with each copy of the manuscript; photocopies are not acceptable. Line drawings should be roughly twice the final printed size. The correct orientation should be indicated if not clear.

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References should be numbered in the order in which they appear, first through the text and then through table and figure legends. The following are examples of ways of writing references.

1. Mukundan, T. and Kishore, K., *Curr. Sci.*, 1991, 60, 355-362.
2. Constantine, G., in *Biology of Bats* (ed. Wimsatt, W. A.), Academic Press, New York, 1970, vol. 1, pp. 319-322.

Acknowledgements should be brief. Footnotes are not allowed except to identify the corresponding author if not the first.

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[*Curr. Sci.*, 1995, 69, 816-821]
-



COVER. The first Indian high-yielding variety, Jaya, released in 1968 for cultivation in irrigated ecosystems. Jaya immediately claimed nation-wide patronage from farmers. See page 438.

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In this issue

Quinine sensitization

Ever since Jesuit priests discovered the anti-malarial properties of the cinchona bark, quinine has been used as a therapeutic for treatment of malaria and as a prophylactic in endemic areas. Although displaced in recent times by synthetic antimalarials, quinine is still important in view of the increased incidence of drug-resistant *Plasmodium* infections, treatment of which will eventually require the availability of diverse therapeutics.

Commenting on a paper published earlier in *Current Science* (Talwar *et al.* 1995, 68, 437-440), Sharma (page 430, this issue) raises an important question on the advisability of using quinine in contraceptive cream formulations and more generally for non-therapeutic purposes. The case of the 'blackwater fever' syndrome is highlighted, where quinine 'sensitized erythrocytes' may become targets for immune attack following *Plasmodium falciparum* infection. Sharma's letter should focus attention on the need to enhance the range of available tools for checking the spread of malaria and more importantly, to consider courses of action for the future, when drug resistance will undoubtedly become a major public health issue. In his response Talwar (page 431) notes that the more recent polyherbal cream formulations contain no quinine at all.

P. Balaram

Calories and longevity

Should one eat less? The answer would appear to be an emphatic yes, if the decision were to be based only on the results reported by Subba Rao *et al.* (page 464). The authors draw an interesting correlation between calorie intake and DNA repair enzyme activities in groups of 'normal' and 'undernourished' human subjects. Diminished consumption appears to result in higher repair enzyme activities (in peripheral lymphocytes), as compared to the control group. Since DNA repair mechanisms are critical for survival of cells and organisms, there have been earlier claims that life span may be related to the capacity to repair DNA damage. This study extends to humans, earlier conclusions based on studies of experimental animals.

P. Balaram

Palaeolake implications

Sediments of the old and the now vanished lakes formed in the later Quaternary epochs unfold eloquent records of the climate changes and the tectonic upheavals that overtook the land 50,000 to 40,000 years ago. The information these palaeolakes provide is valuable for understanding the trend of change in climate and the nature and pat-

tern of earthquakes and resultant landslides. By determining the recurrence intervals of catastrophic natural phenomena, it is possible to attempt prediction of hazards.

T. N. Bagati and his associates (page 479) describe a palaeolake nestling 3600 m above sea level on the northern flank of the Himalaya (Zaskar Range), close to the geological junction of the Indian and Asian continents. They show that the lake originated 45,000 years ago, practically in the same period when many existing and palaeolakes were formed in the Spiti basin in north-eastern Himachal and in Kumaun in the central sector of the Himalaya mountain; as the studies of our group demonstrated. Significantly, the Ladakh lake, like the lakes of Kumaun, evolved as a result of ponding of rivers following tectonic movements of appreciable severity. Another important finding is that the presence of charcoal in the lake sediments, like those of the Kumaun lakes, indicates forest fires resulting presumably from lightning. It seems that forests in dry warm climate caught fire all over the Himalayan domain. The development of warm climatic condition is substantiated by magnetic susceptibility studies. It seems that the whole of the north-western Himalaya was rocked by major earthquakes and attendant hazards some 45,000 years ago.

K. S. Valdiya

Current Science

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We expect that electronic submission will result in quicker processing for publication.

CURRENT SCIENCE

Volume 71 Number 6

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CORRESPONDENCE

Indian journals: To start or scrap?

It is a matter of shame that we tend to publish our findings in foreign journals to get better citation ratings and recognition. It is high time a national consensus was evolved on amalgamating different 'obscure' (*sic*) research journals to form one Indian journal of international standard and repute. This is very essential in the Indian context, notwithstanding the global trend to start highly specialized journals (e.g. *Tissue Engineering*). In India too, university-based journals have bloomed to bridge the scientific community with academia (e.g. *Resonance* from Bangalore) and industries (e.g. *Curie* from Pilani). These journals cater to specific needs at

various levels, and do not fall under the aforesaid category that requires unification.

It is sad to learn that the UGC has decided to abandon for good one such popular science journal, *Chemistry Education*. I have been associated with this journal in two ways: (i) To get handy up-to-date reviews/research information on various aspects of chemistry, during my master's programme at BITS. (ii) As a contributor myself (*Chem. Ed.*, 1995, 12, 19-23).

The editorial board comprised of illustrious names like C. N. R. Rao, D. Balasubramanian, etc. The journal was serving its pedagogic purpose only

too well when the decision to close down has come from the UGC. Considering its immense utility as a supplementary text to University students and instructors, the UGC is urged to continue sponsoring *Chemistry Education*.

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Microbes, molecular biology and the final assault

V. Ramalingaswami's excellent article¹ aptly highlights the infectious disease scenario in the changing world and ends with an optimistic note on the 'art of the possible'. The major instrument for the 'art of the possible' here, obviously, is molecular biology which is increasingly being applied to unravel the molecular underpinnings of the microbial pathogenicity^{2,3}, diagnosis of infectious diseases⁴ and development of newer therapeutic and prophylactic interventions especially novel vaccines. The benefits accruing from these developments would be enormous, but going by their versatility the pathogenic microbes are capable of outwitting all interventional strategies. My obvious reference

is to problems like emergence of new infections⁵, antibiotic resistance and antigenic variations among microbial pathogens. In addition to these problems, extreme complexity of molecular mechanisms underlying microbial pathogenicity puts molecular biologists on an extremely uphill task. Molecular biology as applied to infectious diseases thus needs further impetus. It is probably in this context that Cold Spring Harbor Laboratory⁶ is organizing a meet, later this year, on 'Molecular Approaches to Control Infectious Diseases'. The topics for discussion include - bacterial, parasitic and viral vaccines, HIV/SIV, DNA immunization, immunogenic structures and emerging infections.

Another area which needs particular attention in our endeavours to combat infectious diseases is their epidemiology. In fact, over the years epidemiology has become one of the most neglected aspects of the control of infectious diseases - a fact even acknowledged by CDC⁷, which keeps a tab on the outbreaks of epidemics and emergence of newer infections worldwide. However, recently, European disease-control organizations have joined hands to form surveillance networks to monitor important infectious diseases. CDC is also trying to establish more links across the Atlantic and the Pacific⁷.

To focus attention on the emerging and re-emerging infections, there is a

CORRESPONDENCE

worldwide collaboration among various medical journals in which some of these journals are devoting entire issues to this topic⁸. CDC has even started publishing a journal, *Emerging Infectious Diseases*, to provide peer-reviewed information on emerging microbial pathogens and related issues⁹.

Echoing Ramalingaswami's sentiments – allowing one the luxury of a vision – with further impetus to research on the molecular approaches to the control of the infectious diseases and reinforcement of epidemiological studies, it may be possible to mount a final assault on the conquest of the infectious diseases.

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3. Hormachae, C. E., Penn, C. W. and Smyth, C. J. (eds), *Molecular Biology of Bacterial Infection: Current Status and Future Perspectives*, Society for General Microbiology, Cambridge University Press, Cambridge, 1992.
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8. Clever, L. H., *West. J. Med.*, 1996, **164**, 17.
9. Hughes, J. M., *West. J. Med.*, 1996, **164**, 21–22.

J. S. VIRDY

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The aroma of *Bassia* flower

In an earlier publication (*Curr. Sci.*, 1996, **71**, 257) it was mentioned that 2-acetyl-1-pyrroline (2AP), the aroma molecule of basmati rice and tiger marking fluid may also occur in the flowers of *Bassia latifolia*.

We have now confirmed this with HPLC. 2AP from fresh flowers was extracted as citrate, eluted by paper chromatography and run in HPLC together with standard 2AP-citrate. 2AP occurs in fresh *Bassia* flowers

in relatively large quantities.

S. MIDYA
R. L. BRAHMACHARY

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SCIENTIFIC CORRESPONDENCE

Praneem polyherbal cream for contraception – Safety in malaria endemic countries

WHO reports worldwide 300–500 million clinical cases of malaria, and 1.5 to 2.7 million deaths each year, and about 40% of the world's population is at risk in some 90 countries¹. In India, malaria cases fluctuate between 2 and 2.5 million, and *Plasmodium falciparum* constitutes about 1 million or 40% cases annually. *P. falciparum* is found in almost all parts of the country, but it is a predominant infection in the north-eastern states, Orissa and the forested and irrigated tracts in peninsular India². Furthermore, *P. falciparum* has become resistant to anti-malarial drugs so that recrudescences are more common³.

Praneem polyherbal cream and pessaries with dual properties of contraception and alleviation of genital infections by G. P. Talwar *et al.* have completed phase I clinical trials at the Safdarjung Hospital, New Delhi⁴. It seems to have a go-ahead signal. Praneem polyherbal cream contains purified extract of neem seeds (praneem), quinine hydrochloride and saponins from reetha (*Sapindus mukerossi*), dispensed in a water-soluble cream base. We are concerned at the quinine hydrochloride content in the praneem polyherbal cream which constitutes 30 mg/ml. The recommended dos-

age is 5 ml of the cream each time, i.e. 150 mg quinine hydrochloride.

Classical blackwater fever (BWF) syndrome occurred predominantly in the non-immunes or semi-immune people exposed to falciparum malaria who were taking quinine in an irregular fashion as a prophylactic. BWF used to be a common malaria complication in endemic countries, but with the advent of synthetic anti-malarial drugs such as proguanil and chloroquine, BWF has become extremely rare⁵, although sporadic cases of BWF have now been reported after halofantrine and mefloquine treatment^{6–8}.

Praneem polyherbal cream contraception technology is likely to result in the (i) loss of sensitivity of the malarial parasite to quinine as a result of its low levels in populations⁹ and (ii) polyherbal contraception may trigger immune reaction against drug-sensitized erythrocytes so that people contracting *P. falciparum* infection may come down with BWF, a serious condition characterized by massive intravascular haemolysis leading to anuria and renal failure^{5,8}. At a time when malaria is returning with speed and available malaria control tools have started producing diminishing returns, use of quinine for other than therapeutic purposes may be hazardous.

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one employed for contraception has no quinine hydrochloride (QHCl). Thus the risk, howsoever small, of developing resistance to quinine is not posed.

In reading the results of the experiment reported in Table 1 of the cited paper, Sharma has however, wrongly interpreted the concentrations of QHCl used in the initial prototype formulation. 30 mg/ml was the stock solution, which was tested at various dilutions to determine the dilution at which complete spermicidal action is obtained. In column 3 of the table, it is clearly reported as 3.46 ± 0.30 mg/ml (mean was taken as semen from 10 healthy donors was evaluated). It is not 30 mg/ml, the figure on which Sharma has made projections.

G. P. TALWAR

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G. P. Talwar replies:

The comments of V. P. Sharma are well taken. The polyherbal formulation in use since over a year, which will be the

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Meeting Announcement

24th Annual Meeting of the Indian Biophysical Society and MBU Silver Jubilee Symposium on Structural Biology.

Date: 9-12 December 1996

Place: Indian Institute of Science, Bangalore

Topics include: Proteins and Peptides, Nucleic Acids: Structure and Function, Membrane and Cellular Biophysics, NMR and Crystallography.

Contact: Dr R. Varadarajan
Organizing Secretary, 24th Annual IBS Meeting
Molecular Biophysics Unit
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A new international order on oceans – Indian perspective

M. Sudhakar and B. V. Kumar

The arduous work of the League of Nations for a decade (1973 to 1982) to finalize the text that contained provisions to protect the larger interests of the developing nations has been realized with the coming into force of the Third United Conference on the Law of the Sea on 16 November, 1994. A brief account of the history and enumeration of the events and articles, is attempted here. Recognizing the need to conform to the international law, being a major role player in the very evolution of the new international order of oceans, India amended its Constitution enacting the Maritime Zones Act, in 1976. Realizing that oceans are the next 'techno-strategic frontier' to Atomic Energy and Space, India developed a large institutional framework and launched various challenging programmes at sea, on par with other leading nations on the oceans. The living and non-living resources potential within the country's maritime zones, are discussed.

In the course of time, man is looking more and more towards the sea for substitute material and food. It was believed that a situation would arise wherein the vast expanse of oceans covering 70 per cent of the earth's surface, would be divided into lakes and the more powerful nations would have an unhealthy hold over most of these areas, which are the repositories of both living and non-living resources, that could meet the demands of the world's growing population and rapid industrialization of the developing nations. After the Second World War, mankind realized the importance of a code to regulate the uses of the sea for peaceful and developmental purposes. Although unknown to many, the United Nations made a remarkable achievement in the history of mankind, when the Third United Nations Convention on the Law of the Sea (UNCLOS-III) came into force on November 16, 1994, one year after Guyana brought the total number of ratifications to the required sixty¹.

United Nations Law of the Sea – Retrospect and Prospect

When the League of Nations was replaced by the United Nations in 1945, it was thought desirable to provide for the establishment of a body, i.e. the International Law Commission (ILC) charged with the progressive codification of the International Law of the Sea. ILC submitted a report in 1956 to the UN, which formed the basis for the first UN Conference on the Law of the Sea (UNCLOS-I) held at Geneva, in 1958.

The UNCLOS-I produced a legal framework of rules governing States rights and duties in the Territorial Sea, Continental Shelf and High Seas. UNCLOS-I had adopted four conventions: The convention on the Territorial Sea and the Contiguous Zone; The convention on the High Seas; The convention on the Continental Shelf and a convention on the Fisheries and Conservation of Living Resources of the High Seas. The first three of these have been ratified by a substantial number of States and are based mainly on the customary International Law, as presented in the ILC's report. Consequently, these conventions formed the core of the generally-accepted rules of the Law of the Sea concerning maritime zones.

The major problem which UNCLOS-I faced was to agree on some definite outer limits for the Territorial Sea. Unilateral assertions of coastal states further and further seaward, followed almost immediately; and the interest of naval powers and distant-water fishing nations was aroused. To overcome this, an UNCLOS II was convened in 1960 to discuss the problem, and also the associated question of fishery limits. It failed by only one vote to adopt a compromise formula providing for a six mile Territorial Sea plus a six mile Fishery Zone.

It was agreed in 1970, in the UN General Assembly to convene a third UN Conference, with the task of producing a comprehensive convention on the Law of the Sea (UNCLOS-III). UNCLOS-III had to negotiate a political package that would be acceptable to all States. It had its origin in the Seabed Committee, established in 1967, by the UN General Assembly at the initiative of the then Maltese Ambassador, Arvid Pardo, to examine the implications of a declaration that the deep sea bed lying

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beyond the limits of national jurisdiction is a 'Common Heritage of Mankind'. This involved defining the limits of national jurisdiction, over the sea bed and, therefore, the revision of parts of UNCLOS-I on the Continental Shelf, as well as on the High Seas.

After extensive work spanning about a decade (1973 to 1982), UNCLOS-III was opened for signature in Montego Bay, Jamaica, on December 10, 1982; and was signed by 117 States including India, on the same day. By December 9, 1984, it was signed by 155 States and 4 Entities. UNCLOS-III Regime could not come into force for more than a decade after the Convention was opened for signature. The reasons were many. The main objection came from countries such as the USA, UK, Germany, and other industrialized States; who opposed many of the provisions on seabed mining in international waters (Part XI of UNCLOS-III (ref. 2)). Further, the poor prospects of commercial production of seabed minerals and the lack of economic necessity were the other reasons, for non-implementation of UNCLOS-III.

The Preparatory Commission (PrepCom) was established in 1982 (as per Resolution-I) pending coming into force of the UNCLOS-III. It made commendable progress between 1983 and 1993 and provided a number of fair and viable solutions to the seabed provisions of UNCLOS III. The PrepCom efforts have culminated in a progressive ratification during the last few years, and resulted in the UN Convention on the Law of the Sea (UNCLOS III, 1982) coming into force. On 16 November 1993, the sixtieth instrument of ratification was deposited with the Secretary General United Nations; and the Convention came into force exactly 12 months after, which has a bearing on all States party to it from the midnight of 15 November, 1994.

UNCLOS III (ref. 2) envisages nine different maritime zones with defined and distinct legal status for each zone. The major maritime zones involved in Law of the Sea are shown in Figure 1. Starting from the coast, the nine maritime zones are: Internal Waters; a 12 nm Territorial Sea, a 24 nm Contiguous Zone; a 200 nm EEZ; a Continental Shelf, extending up to the outer edge of the Continental Margin, with outer limits specified; the High Seas beyond the EEZ; the International Seabed Area, beyond the outer limits of the Continental Shelf; Archipelagic Waters; Archipelagic Sealandes; and the Straits used for international navigation. Thus, it seeks to delimit the oceans into a number of maritime zones based on its distance from the shore. The convention also deals with the baselines from which most of these maritime zones are to be measured.

Special provisions were made for the land-locked and geographically disadvantaged countries. Detailed provisions and safeguards on marine environment, marine scientific research, development and transfer of marine technology, and settlement of disputes; are the highlights and new concepts in the Convention. In the wake

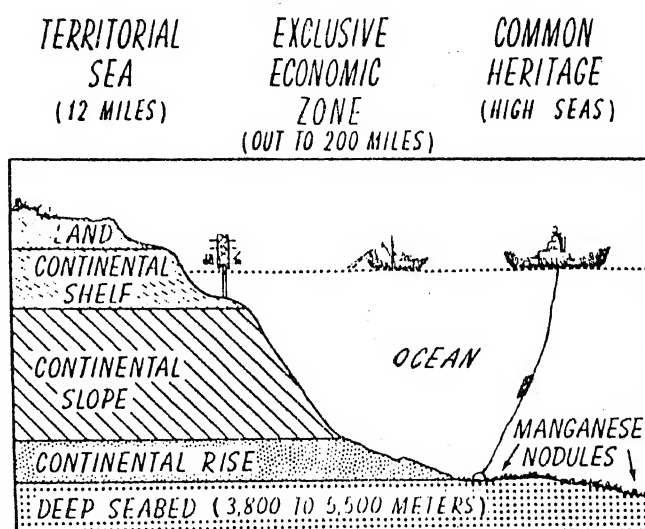


Figure 1. Major maritime zones involved in the Law of the Sea.

of UNCLOS-III coming into force, two UN organizations shall be established, viz. the International Seabed Authority (ISBA) with its Headquarters in Jamaica, to organize and control activities in the seabed area beyond the EEZ, and, the International Tribunal for the Law of the Sea (ITLOS) at Hamburg in Germany to settle the disputes related to Sea.

Every coastal state is entitled to claim these maritime zones. It has sovereignty over the internal waters (i.e. the waters landward of the baselines) subject to innocent passage traditionally used therein; sovereignty in the Territorial Sea and the air above, with the right of innocent passage enjoyed therein by all foreign ships; specific rights in respect of customs, fiscal, immigration and health matters, in the Contiguous Zone, sovereign rights over the living and non-living resources and economic uses of the EEZ, and jurisdiction over marine installations, marine scientific research, and marine environment; sovereign rights over the natural resources of the continental shelf, including sedentary living resources and jurisdiction over certain specified matters.

The High Seas is beyond the jurisdictional limits of Coastal States and is a part of International Waters and in this zone the freedom enjoyed by all is, navigation, overflight, laying of submarine cables and pipelines, construction of artificial islands and installations, and fishing. The seabed under High Seas is the International Seabed Area, and for this, an elaborate regime and an international set-up is provided for in Part XI of the Convention. The International Seabed Area and its resources are the common heritage of mankind and their exploration and exploitation is to be regulated by the Convention.

The Convention lays down that the de-limitation of maritime boundary between States with opposite or ad-

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jacent coasts, shall be in accordance with the median line-cum-special circumstances/historic title role concerning the territorial sea and on the basis of international law in order to achieve an equitable solution in respect of the EEZ and the Continental Shelf.

The Archipelagic State will have sovereignty over its archipelagic waters, with archipelagic sea lanes passage in traditional navigation Straits and innocent passage elsewhere. Like other coastal States, an archipelagic State is also entitled to other maritime zones.

There are other special and specific provisions for the land-locked States, whose right of access to and from the sea, and freedom to transit, is recognized. The land-locked and the geographically disadvantaged States have been given access to the surplus fishery resources of the EEZ in the neighbouring coastal State of the region, and for the conduct of marine scientific research.

A large majority of Coastal States conform to the provisions made in UNCLOS-III on various sea uses and resource utilization and have proclaimed their right to manage and control access to the resources and the use of the sea, and the seabed, up to a distance of 200 nautical miles from their coastlines. Coastal States are also extending their jurisdiction to the same distance in so far as environmental pollution, control of shipping, including supervision of dangerous cargoes and general problems of safety are concerned.

Establishment of International Seabed Authority

The International Seabed Authority situated in Jamaica, is seen in operation, when the UNCLOS came into force on 16 November 1994. Since that time, over two years ago, twenty-five additional states have ratified the Convention¹ (Table 1). India, having ratified the Convention on 29 July 1995, got involved in arduous deliberations and negotiations since then for a place in the Council of the ISBA. A high-level team, headed by A. E. Muthunayagam, had intense negotiations during the State Parties meeting held in New York from March 4 to 8 and again during the first part of the second session of the ISBA at Kingston, Jamaica, beginning March 11 (ref 3). The delegation staked a claim for India under the investor's category based on the country's various plans and activities at sea, particularly the investments made in long-term programmes like 'Polymetallic Nodule Surveys' in the Central Indian Basin, that brought distinction amongst the other developing nations. The election on March 18 entitled India to be a member of the Council for eight years, in two phases – first, for a two-year term from the current year and the next for four years beginning in the year 2000 (ref. 3). The Council consists of 36 members of the Authority elected by the Assembly, the sole and supreme organ of the Authority

Table 1. Details of countries that ratified^{1,*} the UNCLOS III after the 60th instrument of ratification deposited by Guyana in November 16, 1993

Ratification No.	Date of ratification		Name of the Country
61	January	1994	Bosnia
62	June	1994	Comoros
63	July	1994	Sri Lanka
64	July	1994	Viet Nam
65	August	1994	Macedonia
66	October	1994	Australia
67	October	1994	Germany
68	November	1994	Mauritius
69	November	1994	Singapore
70	December	1994	Sierra Leone
71	January	1995	Lebanon
72	January	1995	Italy
73	February	1995	Cook Islands
74	April	1995	Croatia
75	April	1995	Bolivia
76	June	1995	Slovenia
77	June	1995	India
78	July	1995	Austria
79	July	1995	Greece
80	August	1995	Tonga
81	August	1995	Samoa
82	November	1995	Jordan
83	December	1995	Argentina
84	January	1996	Nauru
85	January	1996	Republic of Korea

*Information on ratifications was provided by the United Nations Division of Ocean Affairs and Law of the Sea in New York.

consisting of all the members¹. India had staked a claim and got a seat under the pioneer investor's category based on its various plans and programmes in seabed regime, and the preparatory investments made in polymetallic nodules, which registered India as the Pioneer Investor, with a Pioneer Area in the Indian Ocean. The other competitors for this category were USA, Russia, Japan, Germany, China, France and The Netherlands. China, France and Germany were elected to the Council under the investors' category³.

Establishment of Department of Ocean Development

After Atomic Energy and Space, the Oceans are India's next 'techno-strategic frontier'. Recognizing this, India made commendable progress in developing institutional framework for ocean management since 1978. A Department of Environment was established in 1980. Soon after, appreciating the importance of the ocean sciences, a need was felt by the then Prime Minister Indira Gandhi appreciating the importance of the ocean sciences, to establish a separate department to deal with oceanographic affairs, in the wake of UNCLOS-III coming for

signature. As a result, the Department of Ocean Development (DOD) was established in July, 1981, with the aim of creating a deeper understanding of the oceanic regime of the Northern and Central Indian Ocean and also development of technology and technological aids for harnessing of resources and understanding of various physical, chemical and biological processes. The Ocean Policy was enunciated in 1982.

In order to fulfil the objectives in the Ocean Policy, the Department has been promoting and implementing the following major research and development programmes:

- * Scientific research in Antarctica
- * Exploration and development of technology for exploitation of deep sea bed polymetallic nodules
- * Assessment of living and non-living resources
- * Programmes relating to coastal zone and islands
- * Marine scientific research and development of specialized manpower
- * Development of marine instrumentation and systems
- * Policy relating to Law of the Sea.

Indian perspective

Peninsular India is bestowed with a long coastline of about 7500 km along the Arabian Sea in the West, and the Bay of Bengal in the East, which opens into the

Indian Ocean in the South. It has over 1200 islands comprising the Andaman and Nicobar in the East and Lakshadweep in the Southwest.

A traditionally maritime country, with varied interests in the sea, especially in fisheries, oil and gas, shipping, seabed mining, power generation, protection of marine environment and national security, India participated effectively in UNCLOS-I (1958), UNCLOS-II (1960) and UNCLOS-III (1973–1982). It has amended the Constitution and enacted the Maritime Zones Act in 1976, the Coast Guard Act in 1978, the Maritime Zones of India Act for Regulation of Fishing by Foreign Vessels in 1981; and issued rules in 1982, and the Environmental Protection Act in 1986. Thus, the present Indian legislation on maritime zones largely conforms to the international law. The country has well demarcated and distinct maritime zones such as a 12 nautical mile Territorial Sea, a 24 nm contiguous zone, a 200 nm EEZ, the Continental Shelf that may extend beyond 200 nm (to be demarcated), and a Pioneer Area in the international seabed area for exploitation of polymetallic nodules.

By the year 2000 AD, the Indian population is estimated to be 1021 million, constituting about 16 per cent of an estimated world population of 6351 million⁴. The demand for minerals rises with the population explosion and rapid industrialization. India's demand for mineral resources is expected to increase two to threefold. The growing demand by the turn of the century for some metals awakes in us the desire for further discoveries

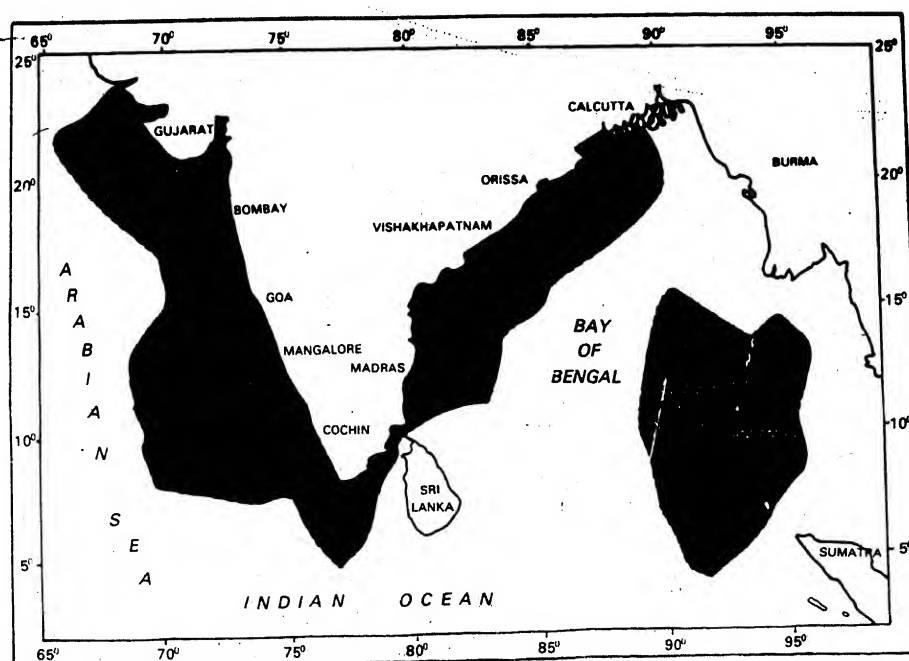


Figure 2. Map showing the Exclusive Economic Zone (EEZ) of India.

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and investments. In addition, the production cost is likely to increase with energy prices and may make some low-grade mineral deposits uneconomical to mine on land. A growing need to look for new deposits of minerals at sea, is felt.

India gains a little over 2 million square kilometres of area under the EEZ regime (Figure 2), and this is equivalent to about two-thirds its landmass. In this area, the exclusive right to utilize living and non-living resources vests with our nation. The seafloor of the continental margins of India is covered by a wide variety of non-living resources. In addition to the vast deposits of offshore sand and gravel mainly used for road and building works, heavy mineral placer deposits containing ilmenite, magnetite, monazite, zircon, and rutile are located and exploited on many beaches and shallow waters along the coasts of Maharashtra, Kerala, Tamil Nadu, Andhra Pradesh, and Orissa. The National Institute of Oceanography (NIO), Goa, carried out exploration for ilmenite placer deposits in the Konkan coast extending offshore to a distance of 2 to 8 km at 18 to 20 m water depth and the estimated reserves are more than 12.5 million tonnes⁴.

Biogenic sediments containing offshore limeshells and sand deposits are investigated from shallow waters of the Laccadive islands (712 million tonnes) and the Gulf of Kutch (2 million tonnes), the outer shelf of Bombay and the Backwaters of Kerala (3 million tonnes)⁴. These deposits can be used by the cement, lime, glass, paper and chemical industries. Efforts are being made to identify the rich deposits of phosphorites along the Western Continental Shelf of India. Phosphorite exploitation from the offshore would make India self-sufficient in producing the raw material for the fertilizer industry. The offshore oil and gas scenario is positive. India's offshore oil and gas has overtaken the land production and supply, and is now double that of the land production. Under the new economic policy the Government of India invited bids for oil exploration in offshore blocks of Bombay, Cauvery Basin and the Krishna-Godavari Basin. Thus, India may enter into joint ventures with foreign companies in the form of production-sharing contracts. A joint venture between British Gas and the Gas Authority of India Ltd (GAIL) is a positive step towards offshore gas development for use in power generation and fertilizer industries. The variety and vast deposits available in our vicinity form a part of our resource, under the new international regime as stipulated in UNCLOS III. India has both sovereign rights and jurisdiction over these resources under the provisions of UNCLOS III.

On the living resource front, India's marine fish catch has been increasing since the declaration of its EEZ in 1977. The annual fish production in the country has touched an estimated 4.78 million tonnes in 1994-95 with the marine catch accounting for 2.69 million tonnes

inland catch reaching 2.09 million tonnes. Against the present estimates of maximum sustainable yield in the EEZ of 3.9 million tonnes, the fish catch accounts for only 70 per cent of the potential. It is, therefore, evident that there is scope for exploitation of the country's EEZ to achieve higher levels of production. In the 50 m depth, about 90 per cent of the assessed potential of 2.28 million tonnes is currently exploited, leaving limited untapped potential. But in depths beyond 50 m, exploitation is only at 40% level, leaving substantial scope for increasing the efforts⁵.

The Fisheries Panel Against Permitting Foreign Vessels, produced a draft National Fisheries Policy, which is awaiting Parliamentary approval. It is against permitting joint venture foreign vessels to fish in the EEZ of the country. The policy which was drafted by an Expert Committee and approved by the Central Board of Fisheries, wants the Government to empower more traditional fishermen to own mechanized boats that would go into the deeper waters for fishing, both surface and bottom dwelling species. Yet India has protected the interests of artisanal fishermen by reserving the 10 km area from the shore and between 10 and 23 km reserved for mechanized fishing vessels and beyond 23 km to be exploited by the industrial fishing vessels. This measure is meant to lower the pressure on the nearshore fishery resources targetted by all country-boats using traditional fishermen. The National Fisheries Policy is aimed at raising the fish production to enable a per capita consumption of 11 kg per annum as against 8 kg per annum at present, during the 9th Plan. It also aims at conservation of aquatic resources and genetic diversity⁵.

The traditional fisherfolk comprise about 2 million population along the coastal belts and they spend most of their lives in fishing. Fishing exports, particularly shrimps exports to Europe, Japan and USA, fetch about 1500 crores in foreign exchange. India is making efforts to develop aquaculture and the Indian Ocean Tuna catch. With assistance from World Bank and the United Nations Development Programme (UNDP), extensive hatcheries are planned throughout the country to distribute seeds for aquaculture development. India is actively involved in developing technologies for aquaculture, cage culture, mussel culture and in the use of satellite remote sensing for locating fish schools in an effort to assist the fishermen in improving their fish catch.

India made a claim in another maritime boundary, too. It made a claim in the International Seabed area for the development and exploitation of mineral resource, viz. 'polymetallic nodules'. Polymetallic nodules, commonly known as manganese nodules, are known to contain appreciable quantities of metals; for example, manganese, nickel, copper and cobalt⁶, that are of strategic significance to India. Nodules occur at water depths of 5 to 6 km in the Indian Ocean. Mining of these minerals

from the Indian Ocean would not only make India self-sufficient in strategic metals but also leave us with surplus metals (for instance, cobalt) for export. India's claim for an 'area' in the Indian Ocean was accepted by the United Nations and on 17 August, 1987, India became the first country in the world to get an area of 150,000 sq km allocated for resource development (Figure 3). As a part of our obligation to the UNCLOS-III, India identified yet another area of the same size in the Indian Ocean (Figure 3), for the International Seabed Authority, to exploit the nodule resources, in order to honour and fulfil the principle of the 'common heritage of mankind'. Later, countries such as Japan, the then USSR and France got their allocations in the Pacific Ocean. This achievement was entirely due to the sustained Indian efforts, especially the scientists from the NIO, since 1981. It would be apt to recall the statement made by the then Prime Minister, Rajiv Gandhi in the Parliament on 26 August, 1987, which is as follows⁷:

'The registration of our claim for a deep seabed mine site indeed provides a concrete indication of indigenous scientific capabilities and achievement. I am sure this House will agree to place on record our appreciation for excellent work done by our scientists and engineers who have taken up the challenging task for exploring new horizons of science to unravel the mysteries of the ocean.'

India has the trained manpower to harness seabed minerals lying at water depths of more than 5 km. Training programmes were organized for the UN personnel and India is developing the skilled manpower required for the International Seabed Authority. Our

research vessels were in the Indian Ocean until recently gathering more information on the exact nature of the seabed and viewing the resources with the help of deep-sea photography. This would lead us to earmark the 'areas' for mining in the near future. The Department of Ocean Development (DOD) is currently formulating long-range programmes on all the maritime zones, especially the legal Continental Shelf regime which needs to be demarcated by India, 10 years from the date of ratification of UNCLOS-III. It is estimated that about 1 million sq km additional area may be encompassed under the legal Continental Shelf of India once the demarcations are made. Further, emphasis is laid on the environmental impact assessment studies to understand the effects of future deep-sea bed mining on ocean dynamics and harmonious living of the biota in the deep-sea region of the Indian Ocean.

South Asia Cooperative Environment Programme (SACEP)

The SACEP formed under the aegis of the Marine Environment Programme of the UNEP to discuss and finalize a Regional Action Plan for the protection of the marine environment in the South Asian Seas Region. It became a legal entity on 7 January, 1982 when nine South Asian countries ratified the Articles of Association of SACEP. In a meeting attended by the plenipotentiaries of India, Bangladesh, Maldives, Pakistan and Sri Lanka, held in New Delhi, on March 24, 1995, the SACEP adopted an Action Plan and the DOD was designated as the Nodal Department⁸. The Action Plan specifies the priority activities including

- (a) Integrated Coastal Zone Management,
- (b) Development and implementation of national and regional oil and chemical spill contingency planning,
- (c) Human resource development through strengthening regional centres of excellence; and
- (d) Control of land-based sources of marine pollution.

In conclusion, the ratification and adoption of the Third UN Convention on the Law of the Sea by an overwhelming majority of nations, including India, and more so by the industrialized nations, established a new international order on the oceans. This should contribute to the sustainable use of oceans for the future good of nations, and to unravel the mysteries of mother nature. This marks a beginning for all nations to put concerted efforts and trust in the UN System, to solve problems related to harmonious living of mankind, and for peaceful use of the natural resources.

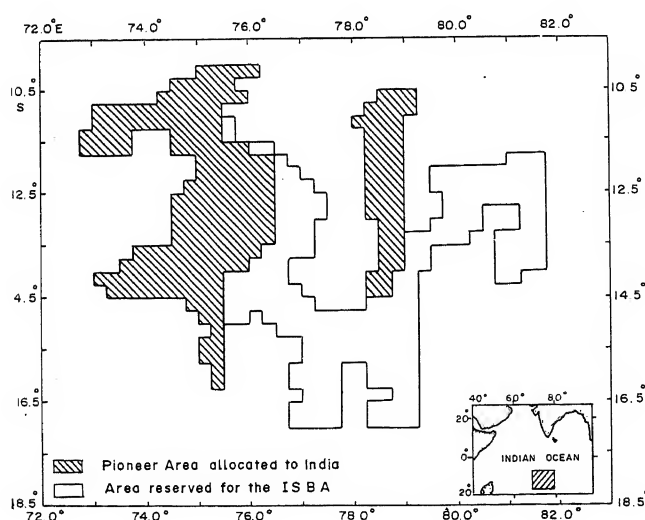


Figure 3. Map showing the two Polymetallic Nodule Areas (150,000 sq km each) in the Central Indian Basin allocated by the PrepCom for the International Seabed Authority on 17 August, 1987.

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Breeding for rice improvement: Where do we stand?

K. Muralidharan, G. S. V. Prasad and C. S. Rao

Rice dominates India's agricultural scene. Rice breeding programmes were started to improve yielding ability, increase efficiency in the use of external inputs and incorporate resistance to biotic and abiotic stresses.

INTRODUCTION of short-statured, non-lodging rice varieties in the mid-sixties led to the green revolution. In rainfed upland, rainfed shallow lowland, semideep water and deep water ecosystems, breeding for rice improvement was aimed at bettering yielding ability, or other traits like maturity duration, resistance to biotic and abiotic stresses or grain quality. From the breeding lines evaluated till 1994, 464 rice varieties have been released for cultivation in India. The grain yield performance data on check varieties and breeding stock, in various co-ordinated multilocal experiments were critically analysed to find the yield gains, if any, in the sequentially released varieties for each ecosystem. Within an ecosystem, there were no significant differences in most of the individual comparison of check variety mean grain yields. A few checks showed low variance and/or low CV, but the variances of the mean grain yields were homogenous. Only a few of these released varieties qualified as rational replacements for the earlier existing checks. Linear regression analyses were performed to find the yield improvement over years in the two floating checks – the mean grain yields of check varieties, both national and local, pooled over all locations tested; and the experimental mean grain yields calculated from yields recorded over all locations by breeding stock

evaluated. The two floating checks showed no yield gain in two decades of rice breeding. The successful incorporation of dwarfing gene in released varieties prevented lodging losses and improved yields harvested in indica rice. Later rice improvement efforts resulted in the diversification of genetic background, maturity duration, grain size and appearance, scent and quality, and incorporation of resistance to biotic and abiotic stresses in varieties released for various ecosystems. Compared to the first released semidwarf varieties in 1966–68, significant yield gain, through exploitation of hybrid vigour or development of new plant type, has not been demonstrated yet. Abandoning of breeding genotypes with similar yielding ability at most centres to fund new initiatives in raising the rice yields are discussed.

Rice dominates India's agricultural scene historically and geographically. Introduction of short statured and input responsive rice varieties during mid-sixties led to the green revolution. The areas under rice cultivation were grouped under five ecosystems: rainfed upland, rainfed shallow lowland, semideep water (< 50 cm depth), deep water (> 50 cm depth) and irrigated. Investments were then made in rice breeding programmes to generate varieties suited to these ecosystems. The aims were to improve yielding ability, increase efficiency in the use of external inputs and incorporate resistance to biotic and abiotic stresses. The multilocal tests of breeding stock developed at dif-

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ferent research centres were organized by the all-India co-ordinated rice improvement project¹. The evaluation of genotype \times environment interactions² in different ecosystems has been the rationale for the multidisciplinary approach to rice improvement research.

Depending on genotype sensitivity to photoperiod, three to four years are needed to identify a promising superior genotype on the basis of data from the multilocal tests³. In the first year, the newly evolved genotypes are tested in replicated local yield trials. The selected breeding lines from these experiments are included in the zonal co-ordinated trials called Initial Variety Trial (or Initial Evaluation Trial). Simultaneously, these breeding lines are also put to screening nursery tests for identifying their reaction to pests and diseases. The breeding lines that yield consistently well for two years are grouped to form Advanced Variety Trials (or Uniform Variety Trials) and tested for two more seasons. Agronomic data on these elite breeding lines are also generated during this period. After a careful scrutiny by different research centres, selected breeding lines are evaluated in on-farm trials for obtaining reaction of farmers and extension workers on the yield performance and acceptability. Considering yield records, agronomic data and the reaction to pests and diseases, candidate breeding lines are identified for release as varieties at the annual workshop by the co-ordinating unit. Each of these elite lines should have at least 10% stable superiority in yield or some special attribute such as disease or pest resistance or quality of the product endowing it with higher profitability or acceptance in the area recommended for its cultivation compared to the existing varieties of the area^{1,3}. A breeding line that fulfils these conditions is then named and released as a new 'high-yielding' variety to cultivators by the state or central variety release committee.

An appropriate comparison base is vital for the successful identification of higher yielding superior genotypes⁴. The traditional base is the standard check—usually a well known released variety that was found good for wide or local adaptability. Check varieties are commonly grown in plant breeding trials for a number of reasons: i) to provide a bench mark for evaluation of other lines⁵; ii) as a series of probe genotypes or interpretational cues to assist in the explanation of response patterns following the classification of all lines⁶; and iii) to aid in the prediction of the performance of new breeding lines in environments not under test, based on prior knowledge gained on the performance of checks in a wider range of environments⁶. In the dynamic rice breeding programme, once genotypes with improvement in yields or in other traits compared to checks are identified and released, some qualify for inclusion as checks. Therefore, a check variety has a variable but nevertheless short life span. The checks are updated frequently

so they do not remain the same over a long time. With each change in the check variety, there is a break in the constancy of comparison over time. In classifying genotypes based on data from plant breeding trials, the mean grain yields of both checks and experimental genotypes across environments are used as subjective choices.

During 1966–1994, up to 14,583 breeding lines have been evaluated nationally under the all India co-ordinated multidisciplinary and multilocal approach to rice improvement. Nearly 2139 elite lines have undergone four years of successful rigorous testing. This led to the identification and release of 464 varieties of rice, either by the states or by the centre⁷. A survey of the crosses developed in 30 breeding programmes showed that breeders gave maximum priority (>93%) to the objective of the improving yield potential⁸. Enhancement in grain yield over time is taken for granted as the target of >10% extra yields as compared to checks is fixed for selection of elite breeding lines for release as varieties and later for a replacement as checks. Over the years several released varieties have replaced the checks in all the ecosystems. The mean grain yields of checks, both national and local, across testing environments over years can be considered to represent a hypothetical floating check of Jensen⁴ that adjusts to yield gains, if any, with each replacement. The experimental mean yields derived from several genotypes across locations denote the base yield level of test breeding stock under different ecosystems and are comparable to another type floating check that adjusts to yields annually.

The aim of this study was to examine 1) whether the sequentially designated check varieties in the co-ordinated experiments were really selected on the basis of yield superiority and stability, and 2) whether there was any grain yield improvement in the two floating checks represented by the check mean grain yields and experimental mean grain yields. To address these objectives, the grain yield performance data on checks and breeding stock in various co-ordinated multilocal experiments under each ecosystem were critically analysed. The results may enable a better understanding on the credibility of the multilocal testing as well as on the benefits from such endeavours.

Materials and methods

Data sets on the performance of breeding lines and checks in 4719 all India co-ordinated experiments^{9,10} were used for this study. These experiments were performed between 1974 and 1994, at 53 locations in 16 states in India under direct-seeded rainfed upland, rainfed shallow lowland, semideep water, deep water and irrigated ecosystems during kharif (rainy) season. All

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these experiments were conducted in randomized block designs with three replications. In rainfed upland experiments, seeds at the rate of 100 kg/ha were sown directly in lines. Every test breeding stock was line sown in 10 rows, each of which was 5 m in length. A spacing of 15 cm between rows or lines was adopted in all these experiments. This direct seeding was also adopted in semideep water and deep water ecosystems, at a spacing of 20 cm between rows and plants. In rainfed shallow lowland and irrigated ecosystems, 30-day-old seedlings were transplanted in experimental plots, at a spacing of 20 cm between rows and

15 cm between plants. The experimental plot size varied with locations; but in most cases the minimum plot size was 10 m². Efforts were made at all locations to ensure crop growth of test breeding stock by adjusting the time of direct seeding or planting, and fertilizer application to suit maturity period or ecosystem where a particular experiment was conducted. Fertilizer rates and insecticide applications were usually decided by the co-operator. In all treatment plots, one border row was excluded and grain from the remaining plants were harvested and expressed as t/ha at 14% moisture.

Table 1. Particulars of checks used in different ecosystems in the all-India co-ordinated rice improvement experiments

Check variety	Cross	Release	Years tested	Locations tested	Experiments
<i>Rainfed upland (direct-seeded) ecosystem</i>					
Bala	TN1/N22	1970	5	12-29	115
Cauvery	TN1/TKM6	1970	14	6-49	321
Ratna	TKM6/IR8	1970	14	7-57	431
Akashi	IR8/N22	1977	8	5-30	117
Annada	MTU15/Waikoku	1987	7	5-13	60
Tulasi	Rasi/Fine Gora	1988	6	5-13	55
Local	—	—	18	5-30	268
<i>Rainfed shallow lowland ecosystem</i>					
Pankaj	Peta/Tongkai Rotan	1969	16	2-25	239
Jagannath	T141 mutant	1969	3	17-24	62
Mahsuri	Mayang Ebos 80/ Taichung 65	1971	5	13-25	82
Savitri	Pankaj/Jagannath	1983	6	4-17	72
Salivahana	RP 5-32/Pankaj	1988	6	2-10	40
Pranava	Vikram/Benong III	1988	4	6-10	32
Local	—	—	18	5-25	233
<i>Semideep water ecosystem</i>					
Tilakachari	Land race	—	4	1-7	19
Utkal Prabha	Waikoku/CR 1014	1983	7	6-10	60
Sabita	Pure line from Boyan	1986	12	5-13	105
Local	—	—	11	3-12	71
<i>Deep water ecosystem</i>					
Jalamagna	Pure line from Badhan	1969	8	2-4	25
Jaladhi 1	Pure line from Kalakher Soul	1981	5	1-3	11
Local	—	—	11	1-4	31
<i>Irrigated ecosystem</i>					
Jaya	TN1/T141	1968	20	3-52	611
Local (m) ¹	—	—	18	3-52	461
Rasi	TN1/Co 29	1977	9	15-57	368
IR 36	IR 1561//IR 244/O. nivara///CR 94-13	1981	6	7-41	158
Vikas	IR 8/TKM 6	1983	5	7-24	109
Local (me) ¹	—	—	18	7-57	561

m = medium duration and me = mid-early duration.

Annada, Mahsuri, and Utkal Prabha are derivatives from *indica/japonica* crosses; Mahsuri was introduced from Malaysia. Jagannath is a mutant. Pranava is a derivative from *javanica/indica* cross. Pankaj and IR 36 were introduced from Philippines. Pankaj, Mahsuri, Jaya, Rasi and IR 36 have been released in many countries.

Data analysis

Details on the varieties used as checks in different ecosystems and the data sets used in this investigation are summarized in Table 1. For each check variety, the mean grain yield in the experiments across locations for each year was calculated. Ecosystem-wise, Bartlett's test of homogeneity of variances of mean grain yields in check varieties was applied¹¹. This is the most widely used and preferred measure as it is applicable whether each variance tested is dependent on the same or different numbers of degrees of freedom. In all possible combinations, comparisons of the mean grain yields¹² of check varieties were made. For each check variety, the coefficient of variation (CV%) in the mean grain yields was also estimated¹³. The CV of a cultivar across environments is considered¹⁴ as a biologically sound measure of its stability.

For each test year in different ecosystems, the mean grain yields for the two floating checks were estimated: 1) mean grain yields of checks, both national and local, were pooled over all locations tested; and 2) experimental mean grain yields were calculated from yields recorded over all locations by breeding stock evaluated. Linear regression analyses were performed to find the yield improvement over years in these two floating checks.

Performance of checks and breeding stock

Rainfed upland ecosystem

Comparisons were made among the mean grain yields recorded by the check varieties (Table 2) evaluated in 1367 experiments in rainfed uplands. Only Ratna exhibited significant differences in the mean grain yields in

comparison with Bala, which was the first released variety for commercial cultivation in rainfed uplands. Bala was denotified and withdrawn from cultivation in 1991 due to grain shattering and loss in harvest in farmers' fields. Ratna recorded highest mean grain yields (4.25 t/ha) under the direct-seeded conditions. The larger variance (0.172) recorded in Ratna was probably due to relatively high number of tests (431). The Bartlett's test showed that variances of mean grain yield of the check varieties were homogenous. Tulasi recorded the lowest variance (0.021) and CV (6.9%) indicating its stability.

During 1976–94, a total of 324 breeding stock was studied. The linear regressions of mean grain yields of the two floating checks in the experiments under rainfed upland ecosystem over time showed a significant decrease (Figure 1). The checks mean grain yields registered a significant decrease from 3.18 t/ha in 1976 to 2.44 t/ha in 1994 ($r = -0.56$). The experimental mean grain yields also registered a similar decrease from 3.22 t/ha in 1976 to 2.44 t/ha in 1994 ($r = -0.58$).

Rainfed shallow lowland ecosystem

Table 3 shows the comparisons made among the mean grain yields recorded by the check varieties evaluated in 760 experiments in rainfed shallow lowlands. Of the seven varieties used as checks, none exhibited any significant difference in the mean grain yields in comparison with Pankaj and Jagannath, which were the first released varieties for commercial cultivation in rainfed shallow lowlands. Jagannath showed sensitivity to photoperiod and was not found to be adapted to all rainfed areas. Therefore, Jagannath, was denotified and withdrawn from cultivation in 1991. Pranava and Salivahana recorded the highest yields. The Bartlett's test, however, showed that variances of mean grain yield of the check

Table 2. Comparison of mean grain yields and variance of mean grain yields among the check varieties in experiments in the rainfed upland ecosystem

Variety	Bala	Cauvery	Ratna	Akashi	Annada	Tulasi	Local
Yield (t/ha)	3.27	2.69	4.25	2.47	2.31	2.11	2.45
Variance	0.112	0.289	0.172	0.071	0.103	0.021	0.230
Bala	—	2.573 ^{ns}	1.534 ^{ns}	1.571 ^{ns}	1.090 ^{ns}	5.470*	2.050 ^{ns}
Cauvery	2.25*	—	1.677 ^{ns}	4.040*	2.805 ^{ns}	14.070**	1.257 ^{ns}
Ratna	4.72**	8.6**	—	2.410 ^{ns}	1.673 ^{ns}	8.390**	1.330 ^{ns}
Akashi	4.75**	1.24 ^{ns}	10.8**	—	1.440 ^{ns}	3.483 ^{ns}	3.214 ^{ns}
Annada	5.02**	1.70 ^{ns}	10.79**	1.08 ^{ns}	—	5.017*	2.230 ^{ns}
Tulasi	7.22**	3.73**	17.1**	3.29**	1.5 ^{ns}	—	11.194**
Local	3.54**	1.28 ^{ns}	11.1**	0.13 ^{ns}	0.74 ^{ns}	1.73 ^{ns}	—
CV(%)	10	20	9.8	11	14	6.9	20
Bartlett's χ^2	11.440 ^{ns}						

Variances of mean grain yields in checks (upper diagonal values represent *F*-values).

Mean grain yields (t/ha) in checks (lower diagonal values represent *t*-values).

*Significant at 5% level; **Significant at 1% level; ns = not significant.

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Table 3. Comparison of mean grain yields and variance of mean grain yields among the check varieties in experiments in rainfed shallow lowland ecosystem

Variety	Pankaj	Jagannath	Mahsuri	Savitri	Salivahana	Pranava	Local
Yield (t/ha)	4.19	4.02	3.96	4.21	4.48	4.52	4.13
Variance	0.263	0.043	0.093	0.264	0.274	0.088	0.175
Pankaj	—	6.076 ^{ns}	2.815 ^{ns}	1.002 ^{ns}	1.040 ^{ns}	2.980 ^{ns}	1.506 ^{ns}
Jagannath	0.56 ^{ns}	—	2.159 ^{ns}	6.087 ^{ns}	6.321 ^{ns}	2.039 ^{ns}	4.036 ^{ns}
Mahsuri	0.94 ^{ns}	0.29 ^{ns}	—	2.820 ^{ns}	2.928 ^{ns}	1.059 ^{ns}	1.870 ^{ns}
Savitri	0.07 ^{ns}	0.60 ^{ns}	0.95 ^{ns}	—	1.038 ^{ns}	2.985 ^{ns}	1.508 ^{ns}
Salivahana	1.17 ^{ns}	1.43 ^{ns}	1.94 ^{ns}	0.90 ^{ns}	—	3.100 ^{ns}	1.566 ^{ns}
Pranava	1.20 ^{ns}	2.46 ^{ns}	2.74*	1.07 ^{ns}	0.13 ^{ns}	—	1.979 ^{ns}
Local	0.39 ^{ns}	0.44 ^{ns}	0.83 ^{ns}	0.39 ^{ns}	1.67 ^{ns}	1.75 ^{ns}	—
CV(%)	12	5.2	7.7	12	12	6.6	10
Bartlett's χ^2	= 4.095 ^{ns}						

Variances of mean grain yields in checks (upper diagonal values represent *F*-values).

Mean grain yields (t/ha) in checks (lower diagonal values represent *t*-values).

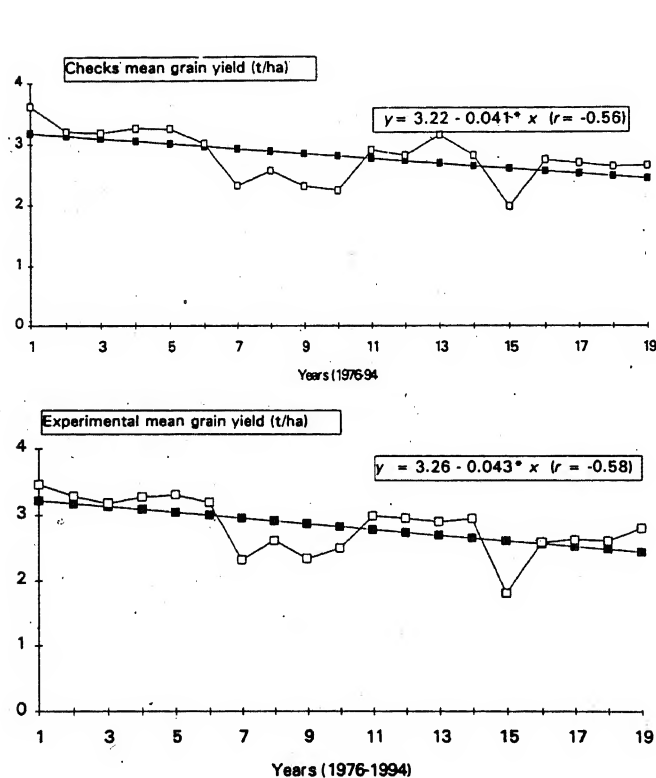


Figure 1. The performance of the two floating checks – check mean grain yields and experimental mean grain yields – across locations over time in the rainfed upland ecosystem. Line fit represents the predicted mean grain yields.

varieties were homogenous. Only Salivahana and Pranava showed low variance and high mean grain yields and therefore qualified for replacement of checks.

During 1974–94, a total of 455 breeding stock was studied. The linear regressions of mean grain yields of the two floating checks in the experiments under rainfed shallow lowland ecosystem over time showed a non-significant increase (Figure 2). The checks mean grain

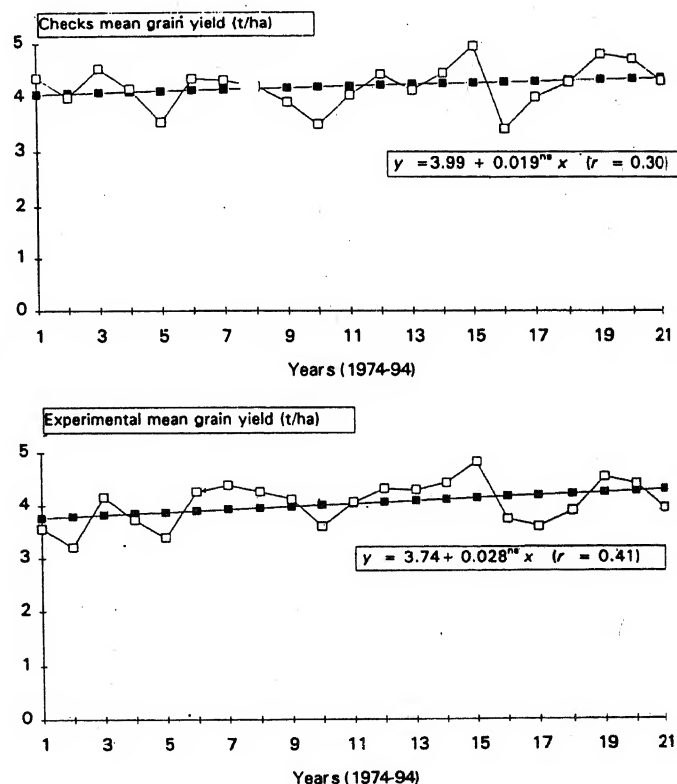


Figure 2. The performance of the two floating checks – check mean grain yields and experimental mean grain yields – across locations over time in the rainfed shallow lowland ecosystem. Line fit represents the predicted mean grain yields.

yields registered a non-significant increase from 4.01 t/ha in 1974 to 4.40 t/ha in 1994. The experimental mean grain yields also registered a similar increase from 3.77 t/ha in 1974 to 4.32 t/ha in 1994.

Semideep water ecosystem

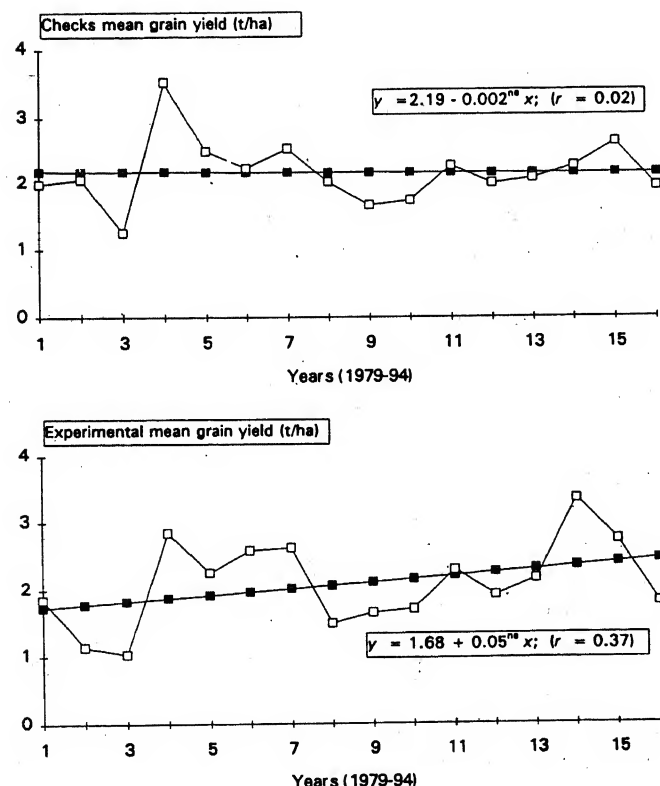
In semideep water ecosystem, comparisons were made among the mean grain yields recorded by the check

Table 4. Comparison of mean grain yields and variance of mean grain yields among the check varieties in experiments in semideep water ecosystem

Variety	Tilakachari	Utkal Prabha	Sabita	Local
Yield (t/ha)	2.26	1.94	2.45	2.19
Variance	0.216	0.125	0.442	0.314
Tilakachari	—	1.72 ^{ns}	2.05 ^{ns}	1.46 ^{ns}
Utkal Prabha	1.31 ^{ns}	—	3.53 ^{ns}	2.51 ^{ns}
Sabita	0.52 ^{ns}	1.89 ^{ns}	—	1.41 ^{ns}
Local	0.21 ^{ns}	1.09 ^{ns}	1.03 ^{ns}	—
CV(%)	21	26	27	18
Bartlett's χ^2	= 2.695 ^{ns}			

Variances of mean grain yields in checks (upper diagonal values represent *F*-values).

Mean grain yields (t/ha) in checks (lower diagonal values represent *t*-values).

**Figure 3.** The performance of the two floating checks – check grain yields and experimental mean grain yields – across locations over time in the semideep water (< 50 cm) ecosystem. Line fit represents the predicted mean grain yields.

varieties evaluated in 255 experiments (Table 4). Of the four varieties used as checks, none exhibited any significant difference in the mean grain yields in comparison to the dominant land race, Tilakachari in semideep water ecosystem. Another land race, Sabita that showed a larger variance presumably due to more tests, recorded

Table 5. Comparison of mean grain yields and variance of mean grain yields among the check varieties in experiments in deep water ecosystem

Variety	Jalamagna	Jaladhi 1	Local
Yield (t/ha)	1.87	1.33	2.2
Variance	0.169	0.371	0.693
Jalamagna	—	2.193 ^{ns}	4.098**
Jaladhi 1	1.908 ^{ns}	—	1.869 ^{ns}
Local	1.146 ^{ns}	2.076 ^{ns}	—
CV(%)	22	46	38
Bartlett's χ^2	= 3.833 ^{ns}		

Variances of mean grain yields in checks (upper diagonal values represent *F*-values).

Mean grain yields (t/ha) in checks (lower diagonal values represent *t*-values).

the highest mean yields. The Bartlett's test showed that variances of mean grain yield of the check varieties were homogenous. Utkal Prabha, a derivative of *japonicalindica*, recorded the lowest variance (0.125) and CV (26%) indicating its stability; but the mean grain yield was lower than the other checks.

During 1979–94, a total of 306 breeding stock was studied. In the experiments under semideep water ecosystem, the linear regressions of mean grain yields of the two floating checks over time showed a non-significant increase (Figure 3). The checks mean grain yields remained nearly at 2.16 t/ha. The experimental mean grain yields also showed a non-significant increase from 1.73 t/ha in 1979 to 2.46 t/ha in 1994.

Deep water ecosystem

Comparisons were made among the mean grain yields recorded by the check varieties evaluated in 67 experiments in deep water ecosystem (Table 5). Of the three land races used as checks, none exhibited any significant difference in the mean grain yields in deep water ecosystem. Local cultivar recorded the highest mean yields. The Bartlett's test showed that variances of mean grain yield of the check varieties were homogenous. There were no significant differences either in the variances or in the yield performances. However, there were quantifiable differences in local cultivars over the other checks. Jalamagna recorded the lowest variance (0.169) and CV (22%) indicating its stability.

During 1984–94, a total of 138 breeding stock was studied in these experiments. The linear regressions of mean grain yields of the two floating checks in the experiments under deep water ecosystem over time showed a non-significant increase (Figure 4). The mean grain yields of both floating checks varied widely.

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Table 6. Comparison of mean grain yields and variance of mean grain yields among the check varieties in experiments in irrigated ecosystem

Variety	Jaya	Local (m)	Rasi	IR 36	Vikas	Local (me)
Yield (t/ha)	4.51	4.57	3.58	3.92	4.18	4.28
Variances	0.225	0.163	0.123	0.446	0.162	0.269
Jaya	—	1.379 ^{ns}	1.824 ^{ns}	1.984 ^{ns}	1.390 ^{ns}	1.196 ^{ns}
Local (m)	0.43 ^{ns}	—	1.323 ^{ns}	2.737 ^{ns}	1.008 ^{ns}	1.651 ^{ns}
Rasi	5.21**	6.22**	—	3.621 ^{ns}	1.313 ^{ns}	2.183 ^{ns}
IR 36	2.42*	2.88**	1.29 ^{ns}	—	2.759 ^{ns}	1.658 ^{ns}
Vikas	1.41 ^{ns}	1.89*	2.91**	0.76 ^{ns}	—	1.664 ^{ns}
Local (me)	1.39 ^{ns}	1.84*	3.63**	1.38 ^{ns}	0.39 ^{ns}	—
CV(%)	11	8.8	9.8	17	9.6	12
Bartlett's χ^2	= 3.778 ^{ns}					

Variances of mean grain yields in checks (upper diagonal values represent *F*-values).

Mean grain yields (t/ha) in checks (lower diagonal values represent *t*-values).

m = medium duration; me = mid-early duration.

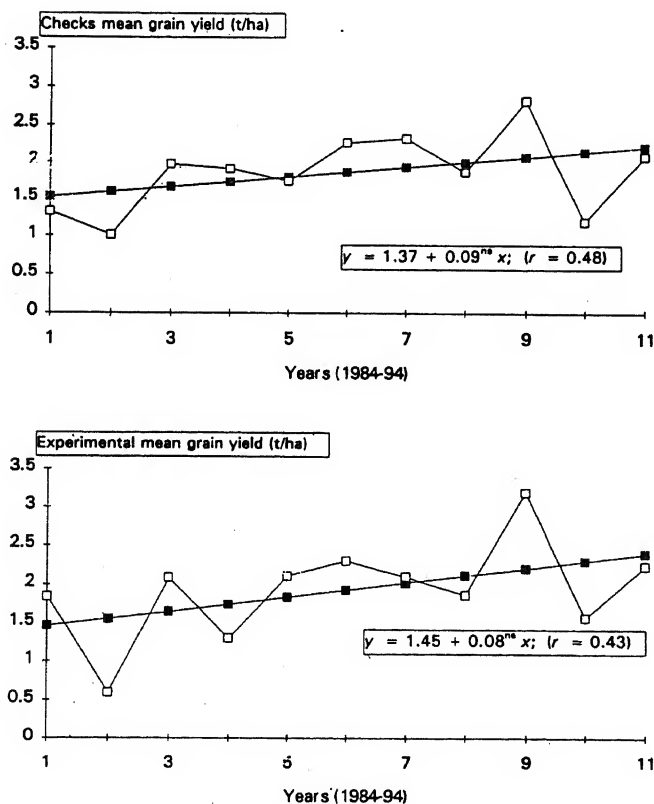


Figure 4. The performance of the two floating checks – check mean grain yields and experimental mean grain yields – across locations over time in the deep water ecosystem. Line fit represents the predicted mean grain yields.

Irrigated ecosystem

Comparisons were made among the mean grain yields recorded by the check varieties evaluated in 2268 experiments in irrigated ecosystem (Table 6). A mid-early

(125 days) and a medium (135 days) maturing local checks were included in these experiments. Only IR 36 and Rasi exhibited significant differences in the mean grain yields in comparison with Jaya, which was the first released variety for commercial cultivation in irrigated ecosystem. Both local checks and Jaya with medium maturity duration recorded the highest mean grain yields. The Bartlett's test showed that variances of mean grain yield of the check varieties were homogenous. Vikas with a mid-early maturity duration recorded the lowest variance (0.162) and CV (9.6%) indicating its stability.

During 1974–94, a total of 916 breeding stock (409 and 507, respectively, in 125 and 135 days maturity groups) was studied. The linear regressions of mean grain yields of the two floating checks in the experiments under irrigated ecosystem over time showed a highly significant positive increase (Figure 5). The checks mean grain yields registered an increase from 3.83 t/ha in 1974 to 4.74 t/ha in 1994 ($r = 0.66$). The experimental mean grain yields also registered an increase from 3.80 t/ha in 1974 to 4.68 t/ha in 1994 ($r = 0.72$).

Yield of breeding lines and varieties claimed as 'high-yielding'

Within an ecosystem, there were no significant differences in most of the individual comparisons of mean grain yields of check varieties. Only a few exceptions were observed. However, the Bartlett's test showed variances in mean grain yields of the check varieties in the different ecosystems to be homogenous. A few check varieties showed stability in grain yields with low variance and/or low CV. Only some of these released varieties qualified as replacements for the earlier existing checks: Akashi (1970), Annada (1987) and Tulasi

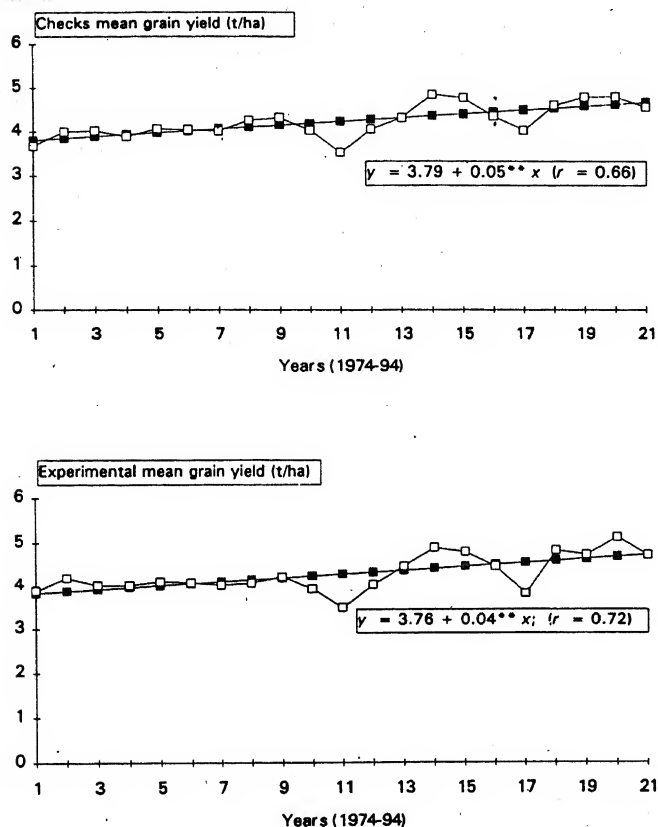


Figure 5. The performance of the two floating checks – check mean grain yields and experimental mean grain yields – across locations over time in the irrigated ecosystem. Line fit represents the predicted mean grain yields.

(1988) for rainfed upland ecosystem; Salivahana and Pranava (1988) for rainfed shallow lowland ecosystem; Utkal Prabha (1983) for semideep water ecosystem; and Vikas (1983, mid-early maturity duration) for irrigated ecosystem. No replacements were found for the checks, Jalamagna (1969) in deep water ecosystem and Jaya (1968, medium maturity duration) in irrigated ecosystem. The strategy of selecting for mean performance from a number of experiments at many locations has resulted in identification of varieties with wide eco-geographical adaptation. As a result of inclusion of one or two checks with good general adaptability, the national test becomes less sensitive for identifying a cultivar's specific adaptability¹⁴. Varieties meeting the criterion of low variance qualify to be named as stable genotypes but are not useful in a breeding programme for maximizing yields¹⁵. There was no consistent increase in the mean grain yields of varieties sequentially designated as checks in the trials for different ecosystems.

The two floating checks, namely, the checks mean grain yields and the experimental mean grain yields, showed no yield gain in two decades of rice breeding. In

fact, in the rainfed upland ecosystem, a significant decrease was discernible in the mean grain yield of these two floating checks. In other rainfed ecosystems, such as shallow lowland, semideep water and deep water ecosystems, there was no significant change in the yield levels, either in the check varieties or in the breeding stock tested over the years. A small but significant yield gain was obtained only in the irrigated ecosystem. This may be attributed to the improved crop husbandry skills and infrastructure development at the experimental locations over these years. In practice it is difficult to separate gains in yield potential in irrigated ecosystem, from the effects of interaction with increased plant density and higher doses of nitrogen. In terms of production gains in farmers' fields, advancement has been only in the non-fragile irrigated and in a part of rainfed ecosystems considered favourable¹⁶. Further, Jaya released in 1969 has remained unconquered for yield potential and continued to dominate as the highest yielding variety till date in the irrigated ecosystem.

In general, elite lines in all these experiments are promoted or discarded after comparisons with the two floating checks, the mean grain yields recorded by the checks and the experimental mean grain yields derived from the yields recorded by all the test lines. If the required condition of a >10% yield superiority¹⁷ in new variety over the previous check was realized in at least some selections, there would have been a consistent increase in mean grain yield in the new releases. If selection for yield in breeding programme has not been effective before inclusion of the newly bred lines in zonal or national trials, then it is not possible to obtain any yield gain at the later stages. Only information on their adaptation is what we achieved from the co-ordinated trials. The 10% numerical superiority claimed for breeding stock can come from either an under-estimation of the check variety or from an over-estimation of the new elite line. This probably led to a nomination of stereotyped breeding stock for evaluation in both national and state experiments. The four years' performance data often comes from a different set of locations, and combined analyses of locations and years are seldom presented when proposing an elite line for release.

Progress in rice breeding

Genotype \times environment interactions determine three major, genetically controlled, physiological components of yield such as the net accumulated biomass, harvest index and harvest maturity duration¹⁸. *Indica* rices are well known to produce high biomass. *Indica* cultivars such as Beria Bhonga produce sturdy culm with less number of tillers per plant. For variations in panicle weight and grain number per panicle also *indica*'s rec-

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ord is unchallenged by 'high-yielding' varieties¹⁹. Traditional *indica* cultivars were, however, prone to lodging and consequent yield losses.

In many *indica* varieties, the overall loss as a result of lodging at pre-flowering stage was estimated at 60% of the grain yields¹⁹. Tall *indica* cultivars fail to express their yield potential in a community due to lodging and mutual shading²⁰. A removal of these inhibitions for estimating their yield potential through propping and wide spacing (50 × 50 cm) led to expression of high yields; with an extra dose of fertilizer (50 kg N/ha) application, the yields increased further. The single plant yields were higher in tall *indica* cultivars, such as Manoharsali (180 g), Handiquesali (180 g), Ngasein (155 g), and Ac 3289 (150 g)²⁰ than dwarf varieties, Jaya and Jagannath (120 g). By supporting tall varieties including *indica* variety, Mtu 15, with bamboo sticks, Jennings at the International Rice Research Institute, Philippines, found that tall varieties yielded essentially as well as did lodging resistant varieties. Moreover the lodging-susceptible varieties when supported, responded well to nitrogen application whereas unsupported plants showed a decided negative response to nitrogenous fertilizers²¹. By the identification and introduction of *DGWG* gene into varieties, non-lodging dwarf stature was achieved with an improvement only in yields harvested. Compared to *indica* land races, the harvest index increased along with a reduction in plant height in the first 'high-yielding' varieties TN 1 and IR 8. Realizing the value of straw in cattle rich rice growing areas, later selections resulted in semidwarf or semitall varieties where aerial biomass was increased. In these semidwarfs, although the harvest index reduced, relatively the maximum grain yields obtained were comparable to the *indicas* and first dwarfs.

The new varieties developed for the different rice ecosystems in the post-green revolution phase have not provided any genetic gains in yield. Exploiting the advantage of multilocal experiments spanning three decades, a number of varieties were released with similar yield potential. However, they differed in other attributes. Varieties with varying maturity duration (early, mid-early, medium, and late) were also developed. In these released varieties, maturity duration, grain size, appearance, scent and quality, resistance to biotic and abiotic stresses could be tailored to suit consumer preferences and needs^{22,23}. Therefore, they replaced the traditional cultivars and the varieties released during the green revolution phase. The periodic release of other cultivars after 1980 resulted in yield stability, at the cost of a marginal reduction in yields. For the different ecosystems, breeding efforts have led to the development of a number of varieties derived from genetically diverse parents but with similar yielding ability. The remarkable diversity attained in rice varieties can be illustrated with

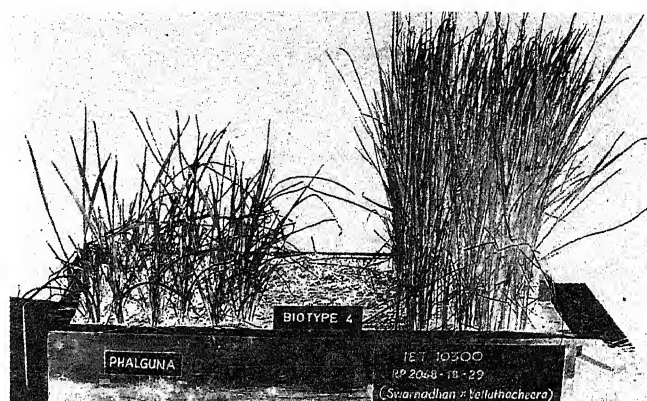


Figure 6. Phalguna – a hitherto resistant variety suddenly showed susceptibility to gall midge (*Orseolia oryzae*) in 1986, after a decade under cultivation in farmers' fields in north coastal Andhra Pradesh. Rice breeding lines developed using diverse genetic sources before a prior knowledge on this new biotype, were tested at Srikakulam district, Andhra Pradesh in 1987. A few elite genotypes from each of the three breeding centres, Directorate of Rice Research, Indira Gandhi Krishi Vishwa Vidyalaya and Central Rice Research Institute, showed resistance like IET 10300 to this new gall midge biotype while Phalguna succumbed²⁴. This remarkable diversity achieved in rice genotypes warrants a reconsideration on maintenance breeding aimed at developing resistant varieties.

the quick success in releasing a variety resistant to a newly-discovered gall midge biotype²⁴. A test of breeding lines developed before a knowledge on this biotype in the area where this new biotype outbreak was observed enabled identification of resistance (Figure 6). Therefore, maintenance research aimed at retaining the levels of resistance considered critical to yield stability^{8,23} is no longer needed in rice improvement programmes¹⁶.

In many crop species, check varieties are normally considered to suffer time erosion as higher yield levels are reached through a genetic yield gain in new breeding lines²⁵. But in this study, results obtained on the performance of checks over time show no evidence of time erosion as there has been no genetic gain in rice varieties developed for specific ecosystems. In each of the ecosystems studied, the checks showed similar yield levels, suggesting that there was redundancy in their replacement. Both national and state rice research centres face critical budgetary shortages. Most breeding programmes operate under tight financial constraints. This demands a rationalization of the size, number and location of rice breeding programmes aimed at generating breeding stock with similar yielding ability. Therefore, abandoning such programmes at most centres would free a substantial resource that could be used in searching for the elusive yield gain.

Avenues for increasing yield

Yield improvement in the successively released varieties of crop species do not show a linear increase²⁵. Rather, the increases have come as steps or a series of small plateau. A yield plateau may exist momentarily, for a certain species in a given region. Usually, each yield improvement to a new small plateau can be associated with the exploitation of a new source of germplasm or trait. Early success of the rice and wheat breeding programme is the result of the definition of a strategy. To ensure maximum progress in a relatively short time, work was concentrated in high-yielding environments defined by irrigation and fertilizer usage. The selection was made for high harvest index types characterized by short stem, erect leaves and photoperiod insensitivity^{26,27}. No yield gains have arisen from selecting for net photosynthesis, nitrogen fixation and utilization, photo- and dark-respiration, light interception, leaf chlorophyll content and enzymes in the pathways of photosynthesis and nitrogen fertilization. Selection for physiological traits will fail to assist breeding for higher yield if their gene actions occur before the actual accumulation of the yield. Wallace and colleagues suggested that failure occurs because the gene activities interact with too many other segregating gene effects. Further, in the physiological processes, the numerous interconnections among parts of the system cause their genetic influence to become unpredictable¹⁸.

Widespread use of single cross corn hybrids after 1960 resulted in a yield jump²⁵. Successful exploitation of hybrid vigour in harvesting higher farm yields in rice has been reported only in China so far. The hybrid rice seed is relatively costly. The induction of male sterility in plants by a dominant chimeric ribonuclease gene²⁸ has demonstrated the possibility of reducing the seed cost. But the yield advantage of hybrids released so far over conventional is a modest 15% (ref. 29). The yield potential of 10 t/ha established by IR 8 and Jaya, the first released semidwarfs²², has not been surpassed so far. In developing high-yielding rice varieties for different ecosystems, the need for a radical change has already been recognized³⁰. Breeding new plant types was initiated during 1989 at the International Rice Research Institute, Philippines, for pushing the yields further. The new plant type with short stature, sturdy stem, panicles with large number of grains (150–200), reduced tillering (5–10 tillers), and erect dark green and thick leaves were developed using the tropical *japonica* germplasm. During the 1994 dry season, 14-day-old seedlings of these elite lines were transplanted at one seedling/hill at different plant densities and fertilized with 200 kg N/ha applied in five split applications. Despite this intensive management, the yields recorded in new plant type advanced breeding lines were not significantly different from the check variety, IR 72 (ref. 31).

In the quest for higher yields, breeding for morphological traits to improve the yielding capabilities of future varieties is appropriate. Valuable yield genes do exist in remote and often unexpected plant materials and successful use of germplasm for improvement of yield could be made. This scope for yield gains by bringing together germplasm from very exotic (completely non-homologous) sources have been demonstrated by the development of triticales³². During 1970–85, research efforts led to realization of increases in total biomass and grains per tiller in wheat. As a result a dramatic increase of 10–20% in grain filling rates occurred in selected lines of *Triticum aestivum*. The reason for this increase is attributed to 1B/1R wheat/rye-derived translocation³³.

Rice germplasm has a rich diversity for the number of panicles per plant, panicle weight, and grain size. Genotypes with over 350 grains per panicle as in Kolomba rices³⁴ such as K 540 and EK 70, or with a test weight of 4.8 g per 100 grains in T 930 from Bengal collection³⁵, have been identified. Grain yield improvements have already been reported in *Triticum durum* from the increased grain number per spikelet³⁶. Most of these traits are supposed to be polygenically controlled. Quantitative genetic models give an insight into the genetic control of a trait. Highly saturated molecular marker linkage maps are available in rice^{37,38}. However, their practical application is still warranted. It is now possible to use these maps to establish associations between marker alleles and genes controlling yield traits. This will enormously decrease the time involved in evaluation. Molecular markers go further, permitting not only determination of the number of chromosomal segments controlling a quantitative trait but also their location on a given chromosome with relative precision. The improved breeding efficiency will be sustained only when aided by good breeding population and their efficient management. New tools are available to the rice breeders for the introduction of specific yield contributing genes into a new plant type. Traits governed by single or a few genes are likely to be inserted successfully. Therefore, new initiatives are needed to identify the required genes in germplasm and deploy such genes in future varieties using skills and modern tools available today for achieving a genetic gain in rice yields.

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PCR-based detection of *Listeria monocytogenes* in dairy foods

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Consumption of dairy foods has been recognized as an important transmission route for human listeriosis, causing health hazards among public. This demands a successful detection assay to declare dairy foods free of *Listeria monocytogenes*, the causative pathogen. Although PCR-based detection of *L. monocytogenes* in dairy foods is associated with problems of PCR inhibition and carry-over contamination, PCR have been applied widely for successful detection of *L. monocytogenes* in dairy foods and found useful for suggesting, monitoring and control measures. Therefore, in the present report, PCR-based detection protocols with special reference to overcome PCR inhibition by dairy foods and culture media constituents over the last 5 years are discussed. Also, critical tips to be kept in mind while performing PCR-based *L. monocytogenes* detection assay are enumerated.

CONSUMPTION of safe food has always been the desire of man. It has now acquired commercial importance with more and more people consuming prepacked food and dairy products. Consequently, the detection of pathogens became cardinal in any centralized food preparation. Although *Listeria monocytogenes* has been recognized as a cause of disease in humans and animals for over 50 years, recognition of listeriosis as an important public health problem dates from the documentation of common source food-borne outbreaks in the last decade¹⁻¹¹ with special risk groups of pregnant women, newborns, elderly and immuno-compromised patients (Figure 1).

Listeria monocytogenes (Table 1) is a human and animal pathogen which is widespread in nature. It is a transient constituent of the intestinal flora excreted by 1-10% of healthy humans. It is extremely hardy and can survive for many years in the cold in naturally infected sources. *L. monocytogenes* has been isolated from a wide variety of foods, including dairy, meat and fish. Most of the food-borne listeriosis outbreaks have been linked to the consumption of dairy foods. Also the evidence that gastrointestinal tract is an important route of infection and that the epithelial cells of the intestine may be primary site of entry for these bacteria has been provided by electron microscopic studies of tissues of in-

fectured guinea pigs¹². It is now recognized that listeriolysin O, a 60-kDa protein, is one of the major virulence factors of the organism. All strains of *L. monocytogenes* are pathogenic by definition although some appear to be more virulent than others.

Besides the above epidemics, *L. monocytogenes* have also been found in dairy foods spanning other parts of the world, viz. Egypt¹³, Singapore¹⁴, United Arab Emirates¹⁵, Scotland¹⁶, Spain¹⁷, Ethiopia¹⁸, Jordan¹⁹, Taiwan²⁰, Italy²¹, Tokyo²², France²³, Poland^{24,25}, Brazil²⁶, New Zealand²⁷, China²⁸, Denmark¹¹, and England and Wales²⁹. The presence of *L. monocytogenes* in dairy foods is mainly due to contamination of raw milk, survival following improper pasteurization, post-pasteurization contamination, resistance to production technologies, contamination during cheese ripening, and survival and growth during product storage³⁰. Therefore, whether the dairy foods are manufactured from raw milk or pasteurized milk, strict detection for *L. monocytogenes* is required to be undertaken.

There is no study available on the incidence of *L. monocytogenes* in India. Since the dairy processing industry in India is growing rapidly as was in the developed world before epidemics, the possibility of such epidemics in India, therefore, cannot be ruled out completely. Consequently, methods for detection of dairy foods-borne *L. monocytogenes* are required for protecting the health of public. Further, questions regarding the epidemiology of the disease, the extent of dairy foods contamination and the importance of the foodborne route of transmission remain unanswered partly due to lack of successful detection assay. A successful assay must be simple, sensitive, specific, reliable, accurate and above all rapid, for detection and identification of *L. monocytogenes* so as to warn against the use of contaminated dairy foods (which have limited shelf life) well in advance. Since an oral infective dose of 10⁵ virulent *L. monocytogenes* is required to cross the intestinal barrier in healthy immunocompetent humans, future studies aiming to determine the potential hazard of *L. monocytogenes* in foods should focus on quantitative rather than qualitative bacteriological aspects³¹.

Various approaches, viz. classical culture, ELISA, DNA probing, miniaturized biochemical assays, etc.

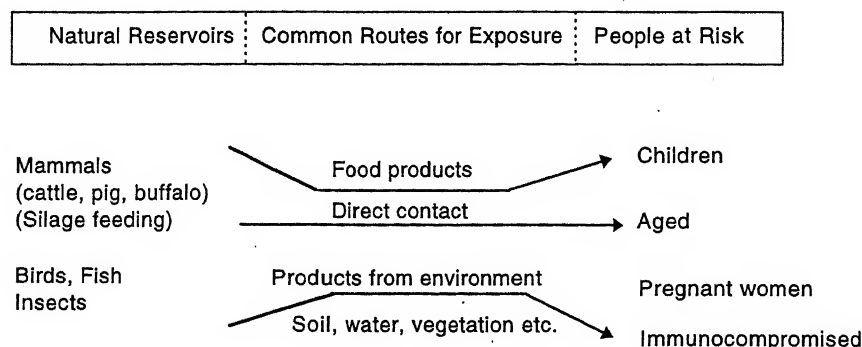


Figure 1. Natural reservoirs, common routes for exposure and people at risk of *L. monocytogenes*.

Table 1. General description of *L. monocytogenes*

Character	Description
General	Rod shaped, 2*0.5 µM, Non spore former, Gram +ve
Motility	Tumbling
Habitat	Soil, vegetation, sewage, silage, water and food
Route of infection	Food and water
Virulence factors	60 kDa, 49 kDa, 40 kDa and 34 kDa
Antibiotic sensitivity	Ampicillin, chloramphenicol, erythromycin, neomycin, tetracycline, cephalosporin

have been used to detect *L. monocytogenes*. The ELISA assay was able to detect *Listeria innocua*, *L. monocytogenes*, *L. murrayi* and *L. welshimeri* after 48 h in artificially contaminated cheese with high sensitivity but without specificity³². A hydrophobic grid-membrane filter (HGMF) colony hybridization method using a digoxigenin-labelled DNA probe is potentially useful for automated detection of the organisms in foods³³. No interference with the present background flora was noticed in a DNA probe assay with the colonies, grown on the selective agar plate, swabbed and washed prior to analysis³⁴.

Approaches used in the past lack one or other criteria of successful detection assay. Standard methods which rely on cultivation of presumptive *Listeria* colonies is time consuming taking 3 to 4 weeks before a species identification is possible³⁵. Therefore, by the time results are available, the product is already consumed. Identification of *L. monocytogenes* by colony hybridization with a specific DNA probe has been reported^{36,37} but here also prior cultivation of the organisms was necessary. A sensitive, quantitative hybridization assay using riboprobes against *Listeria* 16S rRNA has been described³⁸, although the specificity for *L. monocytogenes* was not reported.

The development of primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase, i.e. polymerase chain reaction (PCR)³⁹ and its application to amplify specific fragments of bacterial DNA precede a detection assay which meets all the criteria for successful detection, identification and confirmation of *L. monocytogenes*. Sensitivity of the PCR method for cheese wash-water was higher than classical and the DNA colony-hybridization method⁴⁰. PCR applied to a 2-step enrichment was the most powerful assay for detecting *L. monocytogenes* among selective agar plating, by 'Gen-Probe' DNA hybridization and by the polymerase chain reaction (PCR)⁴¹. With the widespread studies on *L. monocytogenes* in dairy foods, a number of research reports have accumulated over the past 5 years describing various protocols and experimental modifications for its detection.

PCR-based detection assay

PCR in an *in vitro* method for amplification of specific DNA fragments (Figure 2). Two oligonucleotide primers, each complementary to the extremes of opposite strands of DNA separated by a region to be amplified, direct the synthesis of a complementary strand towards each other to produce an exact copy of DNA flanked by primers. Repeating the cycle of 3 independent steps carried out at defined temperatures doubles the numbers, thus increasing the copy number of target DNA in a geometrical fashion (approximately $m2^n$). Here m is the initial copy number of target DNA and n is the number of PCR cycles. The steps involved are denaturation (94–97°C), primer annealing (55–72°C) and extension of annealed primers (at about 72°C) by a thermostable DNA polymerase.

Detection of foodborne microbial pathogens using PCR has been applied widely and reviewed^{42,43}. Denner and Boychuk⁴⁴ developed a species-specific

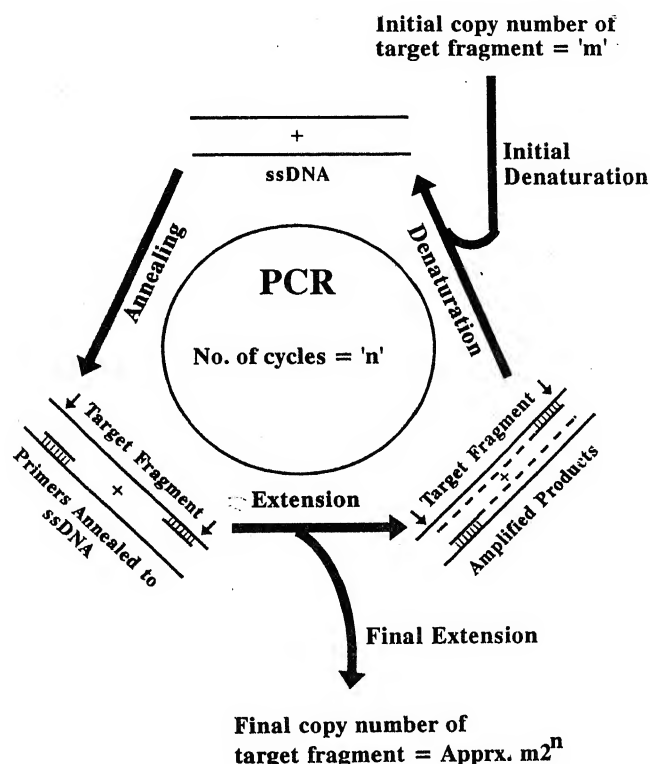


Figure 2. Diagrammatic representation of PCR.

detection assay of *L. monocytogenes* by DNA amplification. Niederhauser and his associates⁴⁵ detected *L. monocytogenes* in food using a PCR-based assay. Since then, a number of research reports have accumulated describing various protocols and experimental modifications for detection of *L. monocytogenes* dairy foods.

In comparison to other methods, PCR offers the following advantages as summarized⁴⁶: (i) a short time requirement improves public health security and minimizes personnel costs; (ii) the method is able to identify microorganisms that are difficult to culture; (iii) the culture and enrichment of pathogens are not necessary for quality control; (iv) PCR reagents are more readily available and easier to store than those required for serological procedures; (v) animal models are not needed; (vi) the choice of primers determines specificity, which contrasts with the fragment cross-reactivities of antisera utilized in immunoassays; (vi) elaborate diagnostic equipment and media are not required, thereby increasing the flexibility as to locations where PCR may be performed and (vii) automated thermocyclers are available.

Principle of the detection assay

Small portion from the sample dairy food is taken for investigation (Figure 3). PCR with *L. monocytogenes*

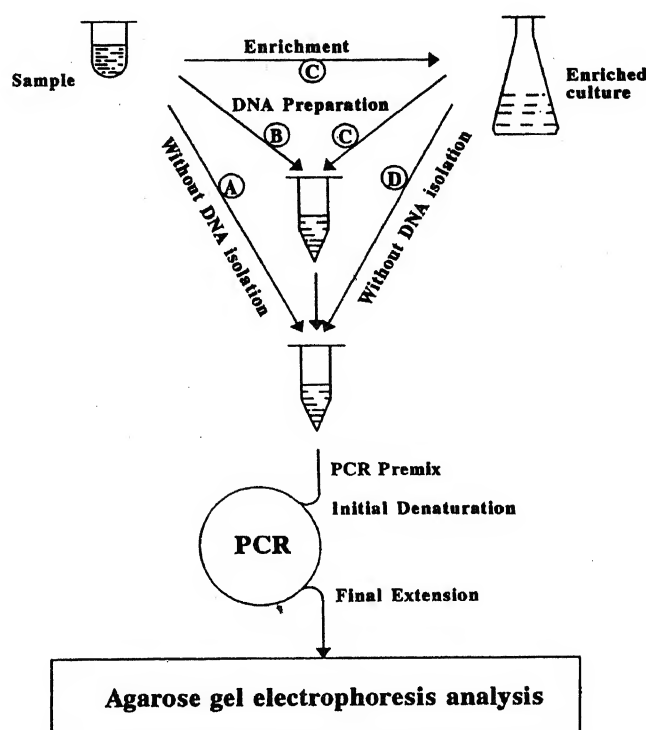


Figure 3. Flowchart for PCR-based detection of *L. monocytogenes* during path: A, without enrichment and without DNA isolation; B, without enrichment and with DNA isolation; C, with enrichment and with DNA isolation and D, with enrichment and without DNA isolation.

specific pair of primers (Table 2) is applied to either (i) DNA isolated with or without enrichment, or (ii) directly to sample or enriched broth to amplify DNA sequences present in *L. monocytogenes* only. The amplified products are resolved on an agarose gel and presence of a specific band signals the sample to be *L. monocytogenes* contaminated. Both positive (isolated template DNA) (PC) and negative controls (NC) (reagents without template DNA) are included to ascertain success of PCR and detect contamination of exogenous DNA respectively.

Reagents

A complete kit as well as individual reagents are available with commercial suppliers and should be used in accordance with manufacturers' instructions. Oligonucleotide primers are specific to each assay employed and can be synthesized in the laboratory or from custom oligonucleotide synthesis facility of commercial suppliers. All components may be premixed, aliquoted and stored in a separate section of the freezer dedicated for PCR reagents located near a laminar flow hood.

Table 2. Primer pairs used in *L. monocytogenes* detection assay

Target gene	Nucleotide sequence from 5' to 3'	Amplified product (bp)	Reference
Listeriolysin O (<i>hlyA</i>)	GCATCTGCATTCAATAAAGA	174	44
	TGTCACCTGCATCTCCGTGGT		
	CGGAGGTTCCGCAAAAGATG	234	62
	CCTCCAGAGTGATCGATGTT		
	ATTGCGAAATTTGGTACAGC	234	63
	ACTTGAGATATATGCAGGAG		
	ATTGCGAAATTTGGTAC	240	50
	CGCCACACTTGAGATAT		
	AACCTATCCAGGTGCTC	267	50
	CTGTAAGCCATTTTCGTC		
	GACATTCAAGTTGTGAA	299	50
	CTGTAAGCCATTTTCGTC		
	GAATGTAACTTCGGCGCAATCAG	388	64
	GCCGTCGATGATTTGAACTTCATC		
	CATAGACGGCAACCTCGGAGA	417	51
	ATCAATTACCGTTCTCCACCATTC		
Dth 18 gene	AACCTATCCAGGTGCTC	520	50
	CGCCACACTTGAGATAT		
	GACATTCAAGTTGTGAA	560	50
	CGCCACACTTGAGATAT		
β -Hemolysin (<i>iap A</i>)	GAAGCACCTTTTACGGAAGC	122	53
	GCTGGTGCTACAGGTGTTTC		
	CCGGGAGCTGCTAAAGCGGT	326	53
	GCCAAACCACCGAAAAGACC		
β -Hemolysin (<i>iap A</i>)	ACAAGCTGCACCTGTTGCAG	131	62
	TGACAGCGTGTGTAGTAGCA		

Primers. Success of detection assay basically depends upon the sequence of primers. The mistakes here will either result in no amplification or non-specific amplification. Availability of nucleotide sequence of target fragment is a must. There are no set rules, but oligonucleotide primers are generally in the range of 18–30 bases in reverse orientation to each other, have similar GC content (>50%), similar T_m , minimal secondary structure (i.e. self-complementarity) particularly in the 3' region (to reduce primer dimer formation), low complementarity to each other, and are specific to target DNA with no cross hybridization to non-target DNA sequences from the same or related species⁴⁷. These are generally designed with the help of a computer program and a number of such programs are available from commercial software suppliers⁴⁸. Some of the primer pairs used in detection assay are given in Table 2.

The optimal concentration of primers to be used in the reaction mixture ranges between 0.1 and 0.5 μ M. Higher primer concentration should be avoided as this may promote mispriming, resulting in nonspecific amplification. Annealing temperatures should be 5–10°C below T_m . However, annealing at higher temperatures for slightly extended times, especially in first few cycles reduces mispriming and helps in increasing the speci-

ficity of the primer pair used in the detection assay. Hot start reduces primer dimer formation⁴⁹.

PCR buffer. Composition of PCR buffer particularly the concentration of Mg^{++} has profound effects on the specificity and yield of amplification. Apparently low Mg^{++} may result due to the presence of EDTA or other chelators in primer or template DNA stocks. Excess of Mg^{++} may result in accumulation of nonspecific products. Therefore, titration of Mg^{++} is highly desirable. Nowadays, PCR buffer without $MgCl_2$ along with a stock solution of $MgCl_2$ is supplied for this purpose (Boehringer Mannheim). Inclusion of Triton-X-100 and/or gelatin has stabilizing effect on enzymes used in PCR and result in better yield. Some recent protocols have recommended the use of 10% DMSO to reduce secondary structures of target DNA.

Deoxynucleotide triphosphates. The dNTPs bind Mg^{++} quantitatively, therefore, dNTPs' concentration in a reaction mixture will determine free Mg^{++} available for enzyme activity. dNTPs are used at 200 μ M final concentration and approximately 50% of dNTPs are left unused after PCR amplification cycles. The pH of the dNTPs stock solution should be neutral. A number of biotechnology companies have come up with stock solution of 100 mM ready-to-use solutions.

Table 3. PCR protocols for detection of *L. monocytogenes* in dairy foods

Sample preparation	Dairy food	Sensitivity	Target gene with amplified product (bp)	PCR parameters	Reference
Direct detection	Inoculated milk	10 bacteria/10 ml	<i>hly A</i> 234 bp, <i>iap</i> 131 bp	D = 30", 95°C; A = 1', 55°; E = 1', 72°C; FE = 5', 72°C; C = 40	62
Direct DNA extraction	Inoculated soft cheeses	10 ³ –10 ⁸ cfu/0.5 g	<i>Dth</i> 112 bp, <i>dth</i> 326 bp	ID = 3', 94°C; D = 1', 94°C; A = 2', 54°C; E = 3', 72°C; C = 30	53
18 h enrichment before DNA extraction	Inoculated yoghurt, skim milk, mature cheese, soft cheese cream	10–100 cfu/g food inoculated with 10 ⁴ cfu/g	<i>hly A</i> 417 bp	ID = 7', 94°C; D = 1', 15", 95°C; A = 30", 62°C; E = 30", 72°C; FE = 1', 72°C; C = 30	51
Overnight enrichment before DNA extraction	–	5–50 cells	<i>hly A</i> 175 bp	ID = 4', 95°C; D = 45", 95°C; A = 45", 60°C; E = 1', 72°C; FE = 5', 72°C, two rounds of 35 cycles	44
Overnight enrichment before DNA extraction	Inoculated pasteurized 2% milk	0.1 cfu/ml of milk	<i>hly A</i> 240 bp, <i>hly A</i> 267 bp; <i>hly A</i> 299 bp; <i>hly A</i> 520 bp; <i>hly A</i> 560 bp	D = 1', 94°C; A = 1', 55°C; E = 2', 72°C; FE = 5', 72°C; C = 36	50
48 h enrichment before DNA extraction	Milk, ice-cream	10 cfu/25 g food before enrichment and 100 cfu/ml of enrichment broth	<i>hly A</i> 388 bp	D = 5', 94°C; D = 1', 94°C; A = 1', 65°C; F = 2', 70°C; C = 30	64

ID = Initial denaturation; D = Denaturation; A = Annealing; E = Extension; FE = Final extension; C = Cycles.

Enzymes. Thermostable DNA polymerase is the enzyme required for amplification and is available with a number of manufacturers. Taq DNA polymerase, the commonly used enzyme in PCR, has been isolated from *Thermus aquaticus*, which has 5' to 3' polymerase activity.

Template DNA. Template DNA used in PCR varies from pure genomic DNA to crude preparation of cells as in dairy foods. Whatever the method employed, the sample should always be taken in either pure sterile triple glass distilled water or 1X PCR buffer.

Protocols

PCR is carried out for amplification of *L. monocytogenes* specific DNA fragment according to the standard procedure as described by Saiki *et al.*³⁹ with modifications to favour optimum amplifications (Table 3). A sensitive and specific method for detection of *L. monocytogenes* in dairy foods consists of culturing samples in listeria enrichment broth (LEB) and subculturing them from LEB to listeria plating media, followed by DNA extraction and species-specific detection of the organism using the PCR⁵⁰. A short enrichment period before PCR amplification allowed detection of the organisms in a range of complex foods contaminated with 104 c.f.u./g, within 24 h or 2 d in soft cheese⁵¹. A novel method employing PCR-coupled ligase chain reaction (LCR) has also been developed for specific detection of *L. monocytogenes*⁵².

The natural samples of dairy foods contain components that inhibit the action of the polymerase enzyme

and necessitate either their specific removal or DNA purification. A considerable decrease of PCR inhibition was obtained by phenol extraction, but, it simultaneously reduced the total amount of DNA which is available for amplification. However, Qiagen columns (prepacked with an ion exchange resin) specifically bind DNA and release this DNA upon elution with a buffer of high ionic strength. Purification of cheese extracts on these columns, in contrast to phenolization, did not lead to excessive loss of DNA in the final preparation⁵³. The direct detection, using PCR of *L. monocytogenes* added to cow milk was also inhibited at some milk concentrations. This inhibitor was moderately heat stable. Inhibition could be prevented by the addition of bovine serum albumin or proteinase inhibitors to the PCR and the evidence suggests that the inhibitor was plasmin⁵⁴.

The polymerase chain reaction detection method, in which the enrichment culture was directly tested, rendered false negative results for 3 soft cheese samples due to the presence of components in the enrichment culture of this cheese that inhibit the PCR⁴⁰. A new detection system – magnetic immuno polymerase chain reaction assay (MIPA) – separates listeria cells from PCR inhibitory factors present in enrichment broths containing food samples by using magnetic beads coated with specific monoclonal antibodies (MAbs)⁵⁵. But in a sensitivity test, PCR was strongly inhibited by cheese components and not by the ingredients of the enrichment broth. Use of DNA purification matrices (DNA Capture Reagent and Geneclean II) may increase the detection limit approximately hundredfold⁵⁶. DNA extracted

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directly from mozzarella cheese artificially contaminated with *L. monocytogenes* samples was amplified by PCR. Amplification was obtained for all samples even at a concentration of 3 cells/g cheese and no interference from natural microflora cheese was observed⁵⁷.

PCR detects both viable and dead *L. monocytogenes*. Therefore, during initial screening with PCR, samples detected positive for *L. monocytogenes* must be tested subsequently using culture methods to confirm for viability of *L. monocytogenes*. Alternatively, PCR protocol may be modified to detect mRNA which represents active and viable cells. Since an oral dose of 10⁵ virulent *L. monocytogenes* is required to cross the intestinal barrier in healthy immunocompetent humans, for a PCR based method to be completely successful, quantitation may also be undertaken in addition to qualitative detection. Perhaps in future, the development of kinetic PCR⁵⁸ and its application to real-time monitoring of DNA amplification reaction run against a concentration standard may provide solution to quantitation problems using PCR.

Analysis of amplified products

The amplified products are resolved on 2–3% agarose gel. The ethidium bromide stain is included in the gel for it increases the clarity of the results as compared to staining the gel after run. The gel is run at constant volts (5–10 V/cm) for 30–70 minutes in 1X TAE buffer and visualized under UV lights. Although the basic setup is same in all cases, the length of amplified fragment is different for each pair of primers. If an expected pattern for PC and NC is obtained, then the assay is valid and there is no contamination. Results can be interpreted accurately. If NC shows PC pattern (due to contamination), then no interpretation is possible.

Avoiding false positives

Since the PCR is a powerful technique to detect even a single copy of target DNA, a false positive may occur if exogenous DNA finds its way to PCR tube. PCR amplifies the target fragment to a large copy number and each fragment is capable of serving as template for the next PCR. Therefore, even if a single copy of amplified product finds its way to the next PCR tube, the detection assay will give a wrong signal. This is called carry over contamination to distinguish it from contamination from naturally-arising DNA. Separate working areas should be allocated to pre-PCR, PCR and post-PCR work. Use of positive displacement pipettes is recommended to avoid pipette barrel contamination. Also, separate sets of pipettes should be allocated for PCR and post-PCR

works. The pipette set used for PCR work should be labelled with red tape and in no circumstances should be used for post-PCR work. Disposable gloves are advised at all the times during PCR set up and frequent change of gloves. All the tubes are closed to avoid air-borne contamination. Given below are the tips which should be kept in mind while performing *L. monocytogenes* detection using PCR^{59–61}.

Pre-PCR work. Prepare reagents in a separate area, premix to 2X stock solutions, aliquot and store these in a freezer section dedicated to store PCR reagents. Record the number of each lot so that if contamination is suspected, it can be traced out easily. Use dedicated chemicals for PCR including water. Autoclave PCR tubes, tips, triple distilled water and mineral oil. Use screw-capped tubes for stocks or tubes that do not require much force to open. This is to avoid splashes and associated contamination. Use siliconized tubes.

PCR-work. Assemble PCR in a clean laminar flow hood having UV lamp. Switch the UV lamp on when the hood is not in use. Expose PCR premix for 5 minutes under UV (254 nm) before assembling PCR in case contamination is suspected. This destroys the template DNA through cross linking whereas primers are safe. Set up positive controls at the beginning and negative control at the end. This represents that the same premix is being added to all tubes and reflects that there was no contamination of premix during reaction set up.

Post-PCR work. This is the area where carry over contamination can arise and maximum care should be taken to prevent spread of carry over contamination. Amplified product containing tubes, gels, tips used for gel loading should be sealed in plastic bags along with a little bleach and disposed off safely. Under no circumstances should these disposables find their way to the pre-PCR area or the PCR area. To prevent 'carry over' contamination from equipment used in post-PCR area, gel apparatus should be soaked in 1 M HCl to depurinate any residual DNA. Surface of the UV transilluminator should be covered with a fresh sheet of plastic wrap for each gel.

Conclusion

Consumption of dairy foods has been recognized as an important transmission route for human listeriosis causing health hazards among public. This demands a successful detection assay to declare dairy foods free of *L. monocytogenes*, the causative pathogen. Various approaches have been used to detect *L. monocytogenes*. PCR offers a successful assay for detection of *L. monocytogenes* in dairy foods. A single copy of specific target DNA is sufficient, enhancing its sensitivity. The assay is rapid enough to provide results on the same day. In addition, it is simple, reliable and accurate. Also, per-

sons without formal education in molecular biology can perform the assay after training. Although the assay is quite easy to perform, it is vulnerable to carry over contamination giving ambiguous results. Therefore, we caution against the spread of contamination. As the methods to distinguish between genuine sample and contamination-related amplification become available, detection assay will be feasible on a single bench without the fear of contamination. In addition, there is need to modify PCR protocols to target mRNA for active and viable *L. monocytogenes* detection sparing dead ones. Also, efforts should be directed towards evolving a quantitative assay.

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Comparative sequence analysis and expression in *E. coli* of the subgroup I-specific antigen VP6 from a G2 serotype human rotavirus IS2

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VP6, the intermediate capsid protein of the virion, specifies subgroup specificity of rotavirus. It is also the most conserved, both at nucleotide and amino acid levels, among group A rotaviruses and is the target of choice for rotavirus detection. In this study we report the sequence of the subgroup I (SGI)-specific VP6 from the serotype G2 strain IS2 isolated from a child suffering from acute diarrhoea in Bangalore and its comparison with the published VP6 sequences. Interestingly, IS2 gene 6 shared highest homology with that from bovine UK strain and the protein contained substitutions by ly-

sine at amino acid positions 97 and 134. In contrast, the amino acids Met and Glu/Asp at these respective positions are highly conserved in all the other group A rotaviruses sequenced so far. These observations have obvious implications for the evolution of serotype G2 and G2-like strains circulating in India. The SGI VP6, of a human rotavirus, possessing epitopes that are conformationally similar to those found in the native protein in the virion, was successfully expressed in *E. coli* and purified for the first time by single-step affinity chromatography.

DIARRHOEAL diseases are the major cause of morbidity and mortality among infants and young children, especially in developing countries. Of the many diarrhoeal agents, rotavirus is the single most important cause of severe, acute infantile gastroenteritis in humans and a variety of domestic animals¹. Rotavirus diarrhoea occurs throughout the year and is estimated to account for about a million deaths annually among young children, thus representing an important public health problem^{1,2}.

In spite of the staggering economic burden, an effective vaccine against rotavirus diarrhoea is yet to be developed.

Rotavirus belongs to the family Reoviridae and consists of a triple-layered capsid³. The outer shell consists of two proteins, VP4 and VP7, the intermediate shell comprises of VP6 and the inner shell is composed of VP2 that encloses a genome of 11 segments of double-stranded (ds) RNA^{3,4}. The genome encodes 6 structural

and at least 5 nonstructural proteins⁵. The outer capsid proteins VP7 and VP4 specify two independent serotype specificities, the G and P types respectively⁶. VP6, encoded by gene segment 6, contains group as well as subgroup-specific epitopes^{6,7}. On the basis of group-specific epitopes, 7 groups A to G have been identified in humans and animals⁵. Group A rotaviruses, the most common pathogens in humans, can be further subdivided into at least 4 subgroups SGI, SGII, SGI + II and non SGI/II on the basis of the SG-specific epitopes⁸. Group A rotaviruses can also be initially characterized as 'short' or 'long' electropherotypes depending on the slower or faster electrophoretic migration, respectively of the dsRNA segment 11 in polyacrylamide gels¹. In general, majority of the human rotaviruses with SGI specificity belong to G2 serotype and exhibit 'short' RNA electropherotype whereas those with SGII specificity have 'long' RNA pattern and belong to other serotypes¹. In contrast, the great majority of animal strains appear to possess 'long' RNA pattern but SGI specificity^{1,9}.

VP6 comprising more than 50% of the virion mass is an important viral antigen and is involved in several viral functions such as replication, transcription and viral morphogenesis^{5,8,10,11}. Although majority of the serum antibodies in the infected host are directed against VP6, anti-VP6 IgG antibodies have not been conclusively shown to be capable of virus neutralization⁸. Recent studies, however, indicate that mucosal anti-VP6 secretory IgA antibodies play an important role in protection against the rotavirus disease¹². VP6 also contains epitopes recognized by cytotoxic T-lymphocytes as well as helper T cells, indicating a role for VP6 in stimulating heterotypic cell-mediated immune response¹³⁻¹⁵. VP6 was shown to function as an excellent immunological carrier for peptides and proteins in vaccine development¹⁶. Because of its high abundance, stability and high degree of conservation among human and animal group A rotaviruses, majority of the procedures for detection of rotaviruses in clinical samples are based on VP6 (refs 7, 17, 18).

Although the SGI-VP6 from a few animal strains had been expressed¹⁹⁻¹², that from a human rotavirus has not been reported. Because of the importance of VP6 in viral diagnosis and to determine the sequence variation in VP6 from Indian rotaviruses, we report here the cloning, comparative sequence analysis and expression in *E. coli* of the SGI VP6 from an Indian G2 serotype strain IS2 isolated from a child with diarrhoea in Bangalore.

Materials and methods

Extraction of viral genomic dsRNA and purification of the RNA segments

Isolation and serotypic characterization of rotaviruses including the G2 serotype strain IS2 isolated from chil-

dren suffering from diarrhoea admitted to various hospitals in Bangalore has been described earlier¹⁸. As IS2 was not adapted to growth in culture, nucleic acids were extracted directly from the clarified supernatants of the 20% suspension of the stool sample as previously described¹⁸. The dsRNA was electrophoresed on a 1% agarose gel in presence of ethidium bromide and the RNA segments 5, 6, 7, 8 and 9 were electroeluted together onto a dialysis membrane and purified by phenol-chloroform extraction²². For identification of gene 6-specific cDNA clones, gene 6 RNA segment was separately purified from the agarose gel.

cDNA synthesis, cloning and identification of VP6 gene-specific clones

In vitro polyadenylation of the denatured dsRNAs, synthesis of cDNA on the oligo(dT)-tailed plasmid (pCDV) primer, construction of cDNA library in *E. coli* HB101 were described previously²²⁻²⁵. The cDNA library, constructed for segments 5 to 9, was screened with ³²[P]-labelled mixed cDNA probe for segments 5, 6, 7, 8 and 9. Gene 6-specific cDNA clones were identified by Southern blot hybridization of the BamHI-digested plasmid DNAs with either the cDNA probe prepared from purified RNA segment 6 or the RNA probe prepared by labelling at the 3' end using *E. coli* poly(A)-polymerase²⁵. Several clones containing inserts ranging in size from 600 to 1500 nucleotides (nt) were identified. Clones OB67 having an insert of 1.0 kb and OB48, OB49 and OB68 containing inserts of 1.5 kb were used for further analysis. Although the reported length of gene 6 from group A rotaviruses is 1356 nt, the observed size of 1.5 kb can be attributed to the presence of poly(dA) and poly(dG)-tails of variable length at the 3' and 5' ends of the cDNAs respectively.

Nucleotide sequence analysis

The complete nucleotide sequence of gene 6 was determined from partial and full length cDNA clones. The cDNA inserts from the original clones were subcloned at BamH I site of pBluescript KS⁺ (pBSKS⁺) vector (Stratagene, CA, USA). From pBS67, subclones were generated utilizing internal sites for restriction endonucleases Nhe I, Pst I and Xba I. Sequence of both strands of the inserts in the subclones was determined using KS, SK and gene-specific primers. Nucleotide sequence was determined by dideoxynucleotide-mediated chain termination method²⁶ using sequenase version 2.0. Sequence near the 5'-end of the inserts in the original clones was determined using Okayama and Berg vector-specific primer. Nucleotide and the deduced amino acid sequences were analysed using version 6.1 of the GCG application software at the Distributed Information

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Centre, Indian Institute of Science. The sequences of the oligonucleotide primers are: 5'-ATCACAACCAGCTCATGAT-3' from nt position 527 to 545; 5'-TTAACTACAGCTACAAT-3' from nt position 675 to 691; 5'-GAAGTGTTACTTCTGCTCT-3' (5' primer for Okayama and Berg vector).

Expression in E. coli and purification of VP6

For VP6 expression, the T7-promoter – and the polymerase – based expression system was used²⁷. As the complete gene 6 cDNA contained 5' and 3' untranslated sequences, the gene 6 ORF was amplified by polymerase chain reaction (PCR)²⁸ using primers corresponding to the 5' and 3' ends of the ORF. The primers contained sites for restriction enzymes of convenience. The sequence of the 5' primer is 5'-GATATCAAGCTTCCCGGGATGGATGTCCTGTACTC-3' and that of the 3' primer is 5'-CTGCAGAAGCTTTTGGACAAGCATGCTTCT-3'. Gene 6 specific sequence is underlined. The PCR-amplified DNA was digested with Hind III and inserted at the Hind III site of pET20b(+) vector²⁷ (Novagen, Madison, WI, USA). The resulting construct is named as pETG6. To express VP6 without the pelB leader sequence, pETG6 was digested with Nde I and Sma I and religated after blunting the Nde I end by Klenow fill-in reaction. Deletion of the pelB leader sequence as well as the stretch of 17 aa downstream of it (upstream of the gene 6 AUG codon) brings the gene 6 AUG codon to within the requisite distance (10 nt in this case) from the Shine-Dalgarno (SD) sequence²⁹. This construct was represented as pETNDG6. *E. coli* HB101 cells were transformed with the ligated DNA and the plasmid DNAs from positive clones were used to transform *E. coli* BL21 (DE3)²⁷. Expression of VP6 in pETG6 and pETNDG6 recombinants was examined by analysis of the cell lysates by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as previously described³⁰. The recombinant VP6 was purified by single-step affinity chromatography using Ni²⁺-NTA-agarose as described earlier²⁴.

Production of polyclonal antiserum and immunoblot analysis

About 500 µg of the recombinant VP6 protein purified from the Ni²⁺-NTA-agarose column was electrophoresed on a 12% preparatory SDS-polyacrylamide gel. The 46.4 kDa band corresponding to VP6 was excised after staining with Coomassie blue and homogenized in 1 ml of phosphate-buffered saline. Polyclonal antibodies were raised by injecting the gel suspension into a New Zealand white rabbit subcutaneously at multiple sites as previously described²². Pre-immune serum collected

from the same rabbit before immunization was used as control.

Purity of the column-purified recombinant VP6 as well as the specificity of the polyclonal antiserum was determined by immunoblot analysis of the protein³¹. Western blots containing either the total cell lysate or the affinity-purified protein were first incubated with either the anti-VP6 antiserum or the SGI-specific mAb 255/60 and then with the secondary antibodies, goat anti-rabbit IgG or goat anti-mouse IgG conjugated with the horse radish peroxidase, respectively. For detection of VP6 by the SGI mAb, the gel was soaked in renaturation buffer (50 mM Tris.Cl pH 7.4, 20% glycerol) for 20 min prior to blotting onto nitrocellulose membrane. The antigen antibody interaction was detected by colour development in citrate buffer containing 3,3'-diaminobenzidine tetrahydrochloride and H₂O₂. The SGI-specific mAb 255/60 was originally produced using rhesus rotavirus (RRV) as the immunizing virus⁷ and provided by Dr Harry B. Greenberg, Stanford University, USA.

Immunoprecipitation

Individual colonies of BL21 (DE3) containing either pETG6 or pETNDG6 were grown overnight in M9 medium. The cultures were then inoculated into fresh sulfate-free M9 medium at 100-fold dilution and grown until the OD₆₀₀ reached 0.4. The cells were then induced with 0.4 mM IPTG in presence of 10 µCi of [³⁵S]-methionine per ml for 10 min at 37°C. The cells were harvested, lysed and inclusion bodies were prepared as described earlier²⁴. The inclusion bodies were dissolved in 0.1 M Tris.Cl pH 8.5 buffer containing 8 M urea by incubating at room temperature for 30 min. Urea was removed from the lysate by centrifugation through a centricon 30 column at 5000 rpm for 30 min. The protein solution remaining in the column was diluted with a buffer containing 10 mM Tris.Cl pH 8.0 and 100 mM NaCl. The process was repeated 3 to 4 times and the final fraction remaining in the column was used for immunoprecipitation. About 50 µl of the protein solution was diluted to 300 µl with RIPA buffer and incubated on ice with 5 µl of mAb 255/60 for 1 h after which 200 µl of protein A-sepharose CL-4B suspension (5 mg of dry gel) was added. The radioactively labelled recombinant VP6 bound to the resin was analysed on a 12% SDS-polyacrylamide gel and the bands were detected by autoradiography as previously described³².

Results

Nucleotide sequence analysis of gene 6 from the symptomatic G2 serotype Indian strain IS2 revealed that the

[illegible]

Figure 1. Comparison of the deduced amino acid sequence of IS2 VP6 with VP6 from other group A rotaviruses. Species origin of the rotavirus strains and the SG specificity of each of the strains are indicated on the left side of the corresponding VP6 sequence. Only the aas that differ from IS2 VP6 are shown. The numbering corresponds to the VP6 from majority of the strains that contain 397 aas. VP6 from the equine strain H2 is 399 aas in length and contains a two-aa insertion at position 296. The IS2 gene 6 sequence was submitted to EMBL database with accession number X94617.

Table 1. Per cent nucleotide and amino acid sequence identities of IS2 VP6 with VP6 from other group A rotaviruses

Rotavirus strain	Species origin	SG	nt	aa
IS2	Human	I	—	—
UK	Bovine	I	94.48	98.74
RF	Bovine	I	93.84	97.73
SA11	Simian	I	87.30	97.23
S2	Human	I	86.74	97.23
1076	Human	I	86.60	98.49
LP14	Lamb	I	85.51	98.24
H2	Equine	nonI/II	82.31	96.47
FI-14	Equine	I/II	79.92	91.44
Gottfried	Porcine	II	79.82	92.44
Wa	Human	II	79.10	91.18
YM	Porcine	I	78.78	90.43

gene was 1356 nt in length. The gene contained a 5' untranslated region (UTR) of 23 nt followed by a long ORF with an AUG codon from position 24 to 26 and a termination codon from position 1215 to 1217. The ORF is followed by a 3' UTR of 139 nt. The ORF codes for a polypeptide of 397 aa with an apparent molecular weight of 44.87 kDa which is similar to that from other group A rotaviruses⁵ (Figure 1). The initiation codon at nt position 24 has the optimal sequence context for a strong initiation codon³³. There is no polyadenylation signal, AAUAAA, in the 3' UTR which is a characteristic of the genomes of the members of the family Reoviridae³⁴.

Comparison of the nt and the deduced aa sequences of gene 6 of IS2 with those of other strains showed a high

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degree of conservation of the SGI VP6 from different strains (Figure 1). The homologies for SGI VP6 ranged from 94.5% to 85.51% at the nt level and 98.74% to 97.23% at aa level (Table 1) with the exception of the porcine Ym VP6. The nt percentage homology with SGII VP6 from different strains was significantly low. Interestingly, IS2 gene 6 shared greatest sequence identity at both nucleotide and amino acid levels (94.48 and 98.74%, respectively) with that from the UK strain of bovine rotavirus and showed less identity (86.74% at nt and 97.23% at aa levels) with the SGI VP6 from the G2 type human strain S2 (Table 1). Majority of the prolines and the three cysteines at positions 96, 197 and 331 are conserved in VP6 belonging to all the subgroups indicating their importance in the proper folding of the protein. The Ile at aa position 56 and Ser at 120 that are conserved in all the SGI and SGI/II VP6 proteins are believed to contribute to SGI specificity³⁵. Ala at positions 172 and 305 that determine SGI specificity are also conserved³⁶ in IS2 VP6. The aa sequences from 58 to 62 (NWNFD) and 159 to 165 (PYSASFT) that determine group-specific epitopes are also conserved³⁷ in IS2 VP6 (Figure 1).

The most striking observation was that the IS2 VP6 contained substitutions by lysine at aa positions 97 and 134 (Figure 1). In all other strains, irrespective of the subgroup, Met at 97 and Glu/Asp at 134 are highly conserved^{35,38} (Figure 1). Also, IS2 VP6 contained a Leu at position 371 in contrast to Ile present at this position in all other group A rotaviruses (Figure 1). The isoelectric point of IS2 VP6, as determined by the programme ISOELECTRIC, was found to be more basic pI (6.68) compared to that of the VP6 from all other group A rotaviruses which ranged from 5.50 to 6.08 with the sole exception of the SGII VP6 from RV3 which had a pI of 7.03.

VP6 was expressed at high level in *E. coli* BL21 (DE3) using the pET20b(+) vector. While an expected 46.4 kDa band was detected in cells transformed with pETNDG6, two polypeptides of molecular weight 50.4 kDa and 48.0 kDa were observed in cells transformed with pETG6 (Figure 2). pETNDG6 lacks the pelB leader as well as the stretch of vector sequence encoding 17 aas preceeding the HindIII site in the vector. Thus the two polypeptides expressed from pETG6 might represent the leader-uncleaved and leader-cleaved forms of the VP6. It appears that the system is overburdened with the recombinant protein and only a fraction of the expressed protein underwent cleavage liberating the 48 kDa VP6 that contained the carboxyterminal six histidines. Inefficient cleavage of the pelB leader has also been observed previously by others³⁹. Alternatively, the 48 kDa species could represent VP6 initiated at an internal AUG codon, most likely that at the NcoI site located downstream of the pelB leader sequence. The fact that only the expected 46.4 kDa protein was expressed

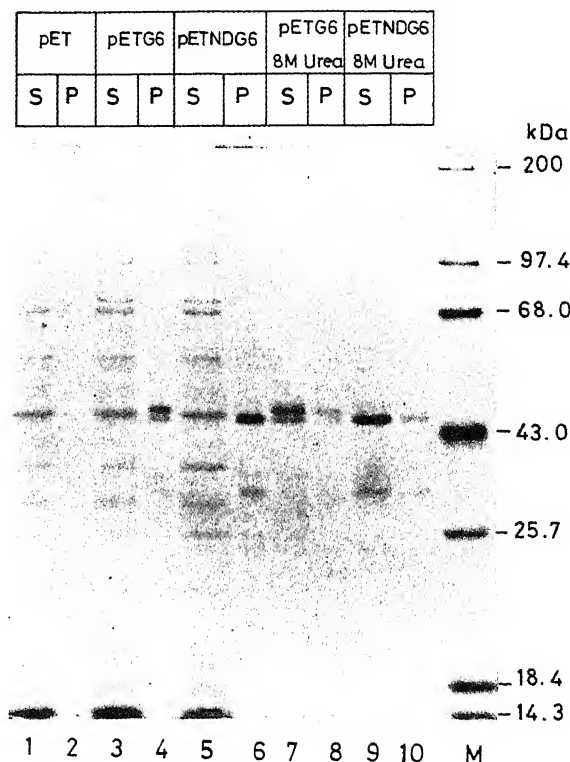


Figure 2. Expression of IS2 VP6 from pETG6 and pETNDG6 in *E. coli*. Soluble fractions of the total cell lysates from cells containing pET, pETG6 and pETNDG6 (lanes 1, 3 and 5, respectively) and the corresponding insoluble fractions (inclusion bodies) of the lysates (lanes 2, 4 and 6) were analysed by SDS-PAGE and the gel was stained with Coomassie blue. Lanes 7 and 9 contain VP6 extracted from the inclusion bodies in 8 M urea and lanes 8 and 10 represent the urea-insoluble fraction. M, protein molecular weight markers. Note that the recombinant VP6 was found only in the insoluble fraction at any time after induction with IPTG.

from pETNDG6 in the absence of the pelB leader sequence strongly suggests that the 48 kDa protein expressed from pETG6 is derived from the 50.4 kDa precursor by cleavage of the leader sequence. The 46.4 kDa protein represented about 12% of the total protein. Clear mobility differences between the 48 kDa and the 46.4 kDa bands could not be detected in this size range in the mini gels used for electrophoretic separation (Figure 2) but mobility differences could be detected when electrophoresis is continued for longer period.

The recombinant protein expressed from either pETG6 or pETNDG6 and purified by affinity chromatography using Ni^{2+} -NTA-agarose resin, upon SDS-PAGE and staining with either Coomassie blue or silver nitrate showed another band of approximately 34 kDa (Figure 3, lane 3 and Figure 4). Silver staining of the gel also revealed another band of approximate size of 24 kDa (Figure 3). These low molecular weight species represent the carboxy terminal half of the protein as they

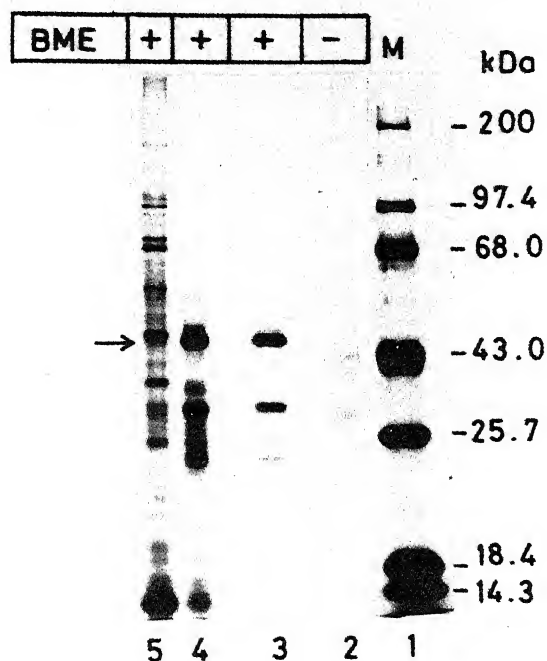


Figure 3. VP6 expressed in *E. coli* forms oligomeric structures. VP6 from the inclusion bodies was solubilized in 8M urea and the urea was then removed by gradient dialysis in presence (lane 3) or absence (lane 2) of β -ME. The proteins were separated by SDS-PAGE after heating at 100°C for 10 min in sample buffer containing (lane 3) or lacking (lane 2) β -ME. The gel was stained with silver nitrate. Lane 4 represents total insoluble fraction and lane 5 contains soluble fraction of the lysate from cells containing pETNDG6. Faint bands of high molecular weight forms of VP6 are seen in lane 2. Also the 24 kDa band is clearly seen in the reduced sample upon silver staining.

contained the histidine tag and could have arisen due to proteolytic cleavage or by internal initiation of translation at Met codons located at nt positions 321 and 552 or 561, respectively.

VP6 expressed in *E. coli* formed inclusion bodies as soon as it was synthesized, probably due to its intrinsic property to form multimeric structures. Studies on induction for varying time periods and with different concentrations of IPTG (0.1 μ M to 0.4 mM) revealed that VP6 expressed at as early as 1 to 10 min of induction was present only in the insoluble fraction. At no stage could we detect VP6 in the soluble fraction of the cell lysate even by immunoblot analysis using polyclonal antiserum.

VP6 from inclusion bodies was examined for oligomeric association and for the presence of the conformation-dependent SGI-specific epitopes. VP6 from the inclusion bodies was solubilized in presence of 8 M urea and the protein was allowed to refold by gradual removal of the urea by dialysing against buffer containing successively decreasing concentration of urea. The soluble protein so obtained was subjected to SDS-PAGE in presence or absence of β -ME (Figure 3). In the ab-

sence of reducing agent, faint bands of higher molecular weight forms corresponding to dimers and trimers could be seen even after heating at 100°C upon silver staining (Figure 3, lane 2), indicating that VP6 expressed in *E. coli* forms oligomeric structures. When the same was not heated (in the presence or absence of β -ME), much of the protein appeared as a smear in the size range between 150 and 80 kDa (data not shown). But upon heating (lane 2) in the absence of β -ME, much of the protein dissociated into the monomer though significant amount also remained in the oligomeric forms. These results suggest that VP6 expressed in *E. coli* existed in different conformational states probably due to rapid formation of insoluble complexes. As observed in the native viral protein and that expressed in insect cells⁴⁰, the recombinant VP6 exhibited anomalous mobility (smaller than the expected 46.4 kDa) as well as heterogeneity in the 46.4 kDa region in the absence of β -ME, suggesting the presence of intramolecular disulphide bridges within the monomer (Figure 3, lane 2).

To determine the authenticity of the protein as well as the presence of the conformation-dependent subgroup I-specific epitopes on the recombinant protein, total lysates in Laemmli buffer were subjected to SDS-PAGE and immunoblot analysis. As shown in Figure 4 B, the recombinant protein was recognized by the SGI-specific mAb 255/60, indicating that the recombinant VP6 contained epitopes that are conformationally similar to those of the native protein in the virion. This is further substantiated by immunoprecipitation of the solubilized radioactively labelled protein with the SGI mAb 255/60 (Figure 4 C). Both polyclonal and monoclonal antibodies recognized the 50.4 kDa, 48.0 kDa, 46.4 kDa and 34 kDa polypeptides while the 24 kDa species could not be readily detected, probably reflecting a lack of all the regions that determine the SGI epitope in this polypeptide (Figure 4 A and 4 B). The intensity of the VP6 band observed in immunoblot and immunoprecipitation analyses (Figure 4, panels B and C) using the SGI-specific mAb 255/60 was not commensurate with the amount of protein used for analysis, reflecting that only a fraction of the VP6 contained conformational epitopes similar to those observed in the native protein.

Discussion

As part of our ongoing studies on the genetic and antigenic variation/diversity in rotaviruses circulating in Indian population, we determined the nucleotide sequence of the SGI-specific intermediate capsid protein VP6 from an Indian strain of symptomatic human rotavirus IS2. As observed in other rotaviruses, the VP6 gene from IS2 is also highly conserved. Comparative sequence analysis revealed that the IS2 VP6 shared greatest sequence identity with the SGI VP6 from the bovine

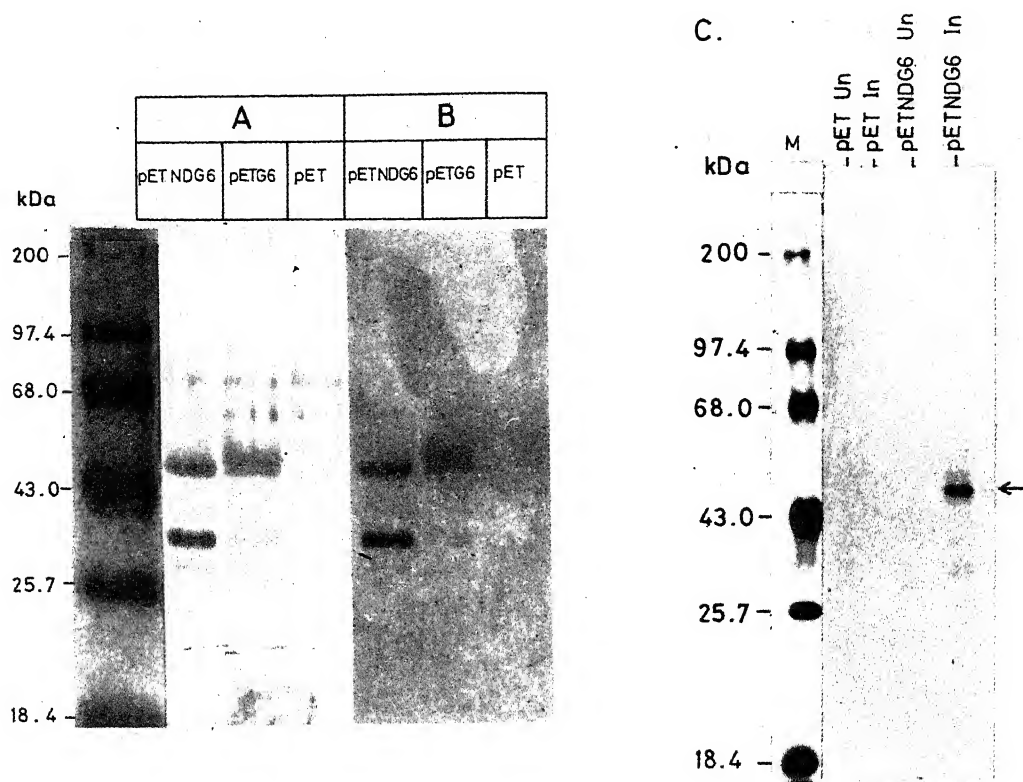


Figure 4. Immunological analysis of VP6 expressed in *E. coli*. Total bacterial cell lysates in Laemmli buffer containing β -ME were electrophoresed on a 12% SDS-polyacrylamide gel. In panel A, the proteins were directly blotted onto a nitrocellulose membrane. In panel B, the proteins were allowed to renature by soaking the gel in renaturation buffer as described in materials and methods. The recombinant VP6 in panel A was detected using polyclonal antiserum raised against the gel-eluted VP6 and in panel B, the SGI-specific mAb 255/60 was used for VP6 detection. Note that the polyclonal antiserum cross reacts with some bacterial proteins migrating above the VP6 band. The small molecular weight 34 kDa protein is also recognized by both polyclonal and monoclonal antibodies. In panel C, the radioactively labelled protein was immunoprecipitated with the SGI-specific mAb 255/60. Un, uninduced, In, induced.

UK strain and exhibited 94.48 and 98.74 per cent nt and aa sequence identities, respectively. Interestingly, IS2 VP6 shared significantly less nt sequence identity (86.74%) with the SGI VP6 from another human strain S2, though the per cent aa sequence identity was 97.23 (Table 1). These observations suggested that the VP6 gene in the Indian IS2 strain of human rotavirus most likely originated from a bovine rotavirus through genetic reassortment in nature. This is not surprising because in earlier studies in our laboratory we have isolated a large number of G10P11 type strains, from asymptomatic neonates in Bangalore and Mysore^{18,41}, that are reassortants between a G10P11 bovine rotavirus and a human rotavirus^{9,18,42}. Recently we have also reported isolation of strains having 'long' RNA pattern and SGI specificity from children with diarrhoea that are likely to have animal origin but distinct from the G10P11 type asymptomatic strains⁴¹. A G9P11 type asymptomatic reassortant rotavirus has also been reported from New Delhi⁴³. Thus it is possible that IS2 and the related serotype G2 and

G2-like strains circulating in India⁴¹ are also reassortants in which the VP6 gene is derived from a bovine rotavirus.

Substitutions by lysine at aa positions 97 and 134 in IS2 VP6 are interesting in the context of the reported substitution by acidic amino acids at several positions in IS2 VP4 (ref. 22). These complementary mutations in IS2 VP6 and VP4 might be of evolutionary significance in stabilizing the interaction between these two proteins in the virions of G2/G2-like Indian strains. Moreover, the presence of clusters of conserved basic amino acids in VP6 from aa position 100 to 154 suggests a functional role for this region.

Though the SGI VP6 from the simian virus SA11 was expressed in *E. coli* as a fusion protein, in insect cells as well as by recombinant vaccinia virus in MA104 cells, expression of VP6 from a human rotavirus has not yet been reported. Using the pET expression system, we have expressed the IS2 VP6 in nonfusion form at high levels and purified by single-step affinity chromato-

phy using Ni^{2+} -NTA-agarose as the matrix. The observation that the recombinant VP6 expressed in *E. coli* formed inclusion bodies as soon as it was formed is not surprising from the fact that majority of the VP6 expressed in insect cells was also found to form insoluble complexes^{40,44}. One of the reasons for the accumulation of VP6 expressed from pETG6 in the unprocessed form could be the nonavailability of the protein for processing due to aggregation as soon as it was synthesized. Alternatively the system is limited to process large amounts of the recombinant protein.

Native VP6 exists as trimer³⁸. There were conflicting reports on the nature of interactions between the monomers in the trimeric form of VP6. Gorziglia *et al.*³⁸ reported involvement of hydrophobic and charge interactions between the monomers in the VP6 trimer in the virion and disulphide bonds in intramolecular organization and hexamer formation. But Estes *et al.*²⁰ reported existence of disulphide bonds between the monomers in the trimer formed from the VP6 expressed in insect cells which was similar to the native VP6 in biochemical and immunological properties. In the present study, VP6 expressed in *E. coli* was found to be capable of forming oligomeric structures (dimers and trimers) and to possess both inter and intramolecular disulphide bridges as observed by Estes *et al.*²⁰.

Although VP6 represents the intermediate capsid protein, it is the major antigen of rotavirus and infected individuals have high-levels of antibodies against VP6. This is probably due to the fragility of the outer capsid resulting in the exposure of VP6 as well as the high abundance of VP6 in the virion compared to other viral proteins. Thus VP6 represents ideal candidate antigen for detection of rotaviruses in clinical samples. Coexpression of VP6 with VP2 or VP7 in the presence or absence of VP4 in insect cells has been shown to result in the formation of double-layered and triple-layered virus-like particles (VLPs), respectively, which are similar to the native particles and have been proposed as candidates for vaccine development⁴⁵. VP6 being an excellent immunological carrier¹⁶, availability of VP6 in large quantities should facilitate its use in vaccines as well as studies on the structure and mapping of the domains of interaction with the spike protein VP4.

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RESEARCH COMMUNICATIONS

Undernutrition and aging: Effects on DNA repair in human peripheral lymphocytes

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Subjects of Indian population belonging to 3 age groups – young (8–14 yrs), adult (20–35 yrs) and old (≥ 55 yrs) were divided into 'normal' and 'undernourished' groups based on Body Mass Index (BMI) and history of diet consumption. DNA repair markers like unscheduled DNA synthesis (UDS), activities of DNA polymerase β and two endodeoxyribonucleases, (UV- and AP-DNases) were studied in the lymphocytes of these subjects under different conditions. The 'undernourished' group showed higher activities of these enzymes and also a reduced decline in age-related DNA repair capacity. These results provide evidence for beneficial effects of reduced calorie consumption in humans as well.

THE integrity of DNA, maintained by a number of DNA repair systems, is essential for the survival of cells and organisms^{1,2}. Numerous physical and chemical factors damage DNA *in vivo* with their origin being both endogenous and exogenous. The resultant DNA damage has been associated with various biological end points, including cancer, mutation, birth defects, aging and other age-associated diseases³. A positive correlation has been claimed between maximum life span and the capacity to repair UV induced DNA-damage, both across species^{4,5}, as well as within and among closely related species^{6–9}.

In a different direction, dietary restriction (DR) is the only environmental paradigm that has been demon-

strated to increase maximum achievable life span in a variety of species^{10–13}. DR has also been reported to modulate the rate or eliminate the occurrence of almost all age-associated degenerative diseases. Furthermore, it is well documented that DR reduces the incidence of both naturally occurring and induced tumours^{14–19}.

It is possible that the mechanism behind the beneficial effects of dietary restriction is positive affectation of DNA repair potential by that regime. Reports, from this laboratory as well as from elsewhere, have indicated that DR does lead to improved DNA repair capacity in experimental animals^{20–22}. However, such effects are yet to be demonstrated in humans. We have therefore, taken up a study on healthy subjects (both sexes) of Indian population living in their natural conditions, with no familial history of organic defects and premature deaths, and belonging to 3 age groups, young (8–14 yrs), adult (20–35 yrs), and old (≥ 55 yrs). At each age, the subjects were divided into two groups of 20 numbers each: one group referred to as normal (NBMI) in which the individuals with a Body Mass Index (BMI)²³ of around 20 or more are included. The other group consisted of individuals with a low BMI of 18 or less (LBMI). Care is taken to see that the range is 16–18 with only a few subjects (5 out of 60) showing marginally lesser than 16.

Table 1 a. Average BMI of experimental subjects

Age	Average BMI	
	N	UN
Y	22.5 \pm 1.3	16.5 \pm 0.5
A	21.5 \pm 1.8	17.1 \pm 0.7
O	23.0 \pm 2.2	17.0 \pm 0.7

Subjects, based on their BMI, were categorized as 'normal' (BMI ≥ 20) and 'undernourished' (BMI ≤ 18). The average BMI of the subjects (20 nos) in the various age groups are shown, where N = Normal (NBMI) and UN = Undernourished (LBMI). Y – subjects grouped as 'young' (8–14 yrs); A – subjects grouped as 'adult' (20–35 yrs); O – subjects grouped as 'old' (≥ 55 yrs).

Table 1 b. Clinical parameters studied in experimental subjects

		Serum cholesterol (md/dl)	Blood haemoglobin (g%)	Random blood sugar (mg/dl)	Serum total protein (g/dl)	Serum albumin (g/dl)	Serum globulin (g/dl)
Y	N	155.2 ± 25.2	12.0 ± 1.1	83.0 ± 10.9	6.9 ± 0.53	4.3 ± 0.62	2.6 ± 0.43
Y	UN	145.8 ± 22.5	11.8 ± 0.4	83.0 ± 12.7	6.9 ± 0.48	4.1 ± 0.51	2.7 ± 0.50
A	N	180.0 ± 27.2	14.5 ± 1.3	76.0 ± 11.5	6.7 ± 0.79	4.6 ± 0.91	2.5 ± 0.45
A	UN	150.2 ± 26.2	13.6 ± 0.8	81.0 ± 27.0	6.5 ± 0.77	4.3 ± 0.93	2.3 ± 0.64
O	N	171.5 ± 26.8	13.8 ± 0.4	79.0 ± 14.0	6.5 ± 0.57	4.0 ± 0.43	2.4 ± 0.50
O	UN	163.0 ± 33.8	13.0 ± 1.4	82.0 ± 31.4	6.7 ± 0.98	4.2 ± 1.10	2.4 ± 0.60

Clinical parameters studied in experimental subjects were Hematology: Total leukocyte count, differential leukocyte count, total erythrocyte count, packed cell volume, blood hemoglobin; Biochemistry: Random blood sugar, serum cholesterol, serum total proteins, serum albumin, serum globulin; Others: Hepatitis B surface antigen detection, chest X-ray, electro-cardiograph. The mean values of clinical examinations of 20 individuals in each group are given. The differences, if any, between any two groups were not found to be statistically significant, where Y = young, A = adult, O = old, N = normal (NBMI), UN = undernourished (LBMI).

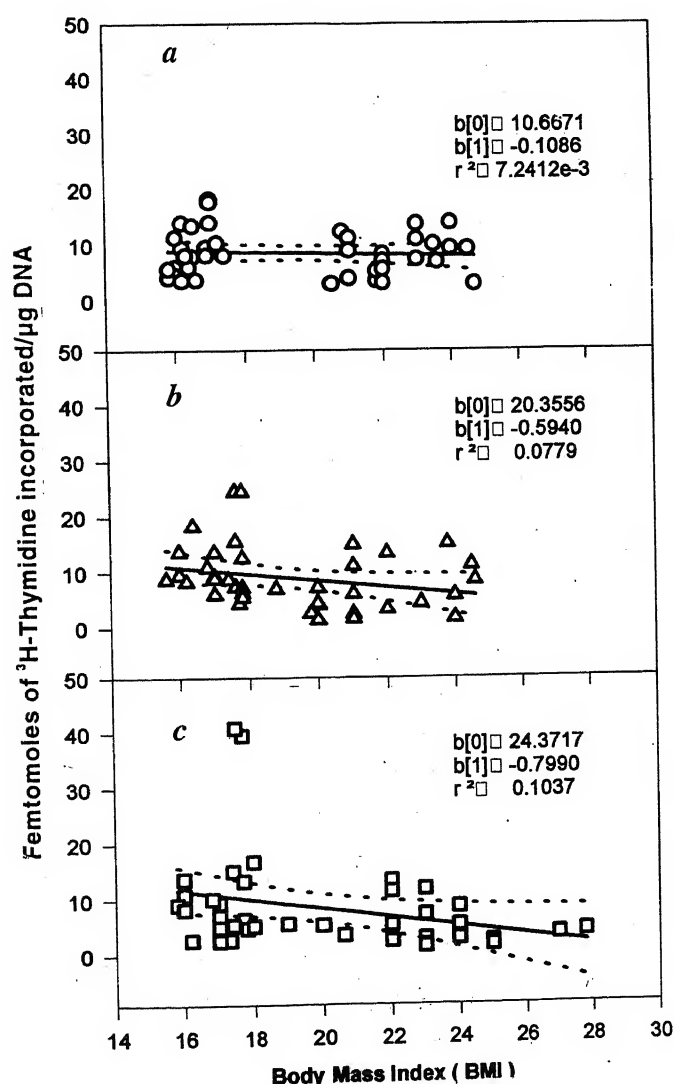


Figure 1. Basal DNA repair in peripheral lymphocytes of human subjects as a function of Body Mass Index at young (a), adult (b) and old (c) ages. Linear regression analyses are shown inside plot-box; $b(0)$ = intercept on y-axis; $b(i)$ = slope of the regression curve; r^2 = regression coefficient. At each age, 40 independent values are plotted.

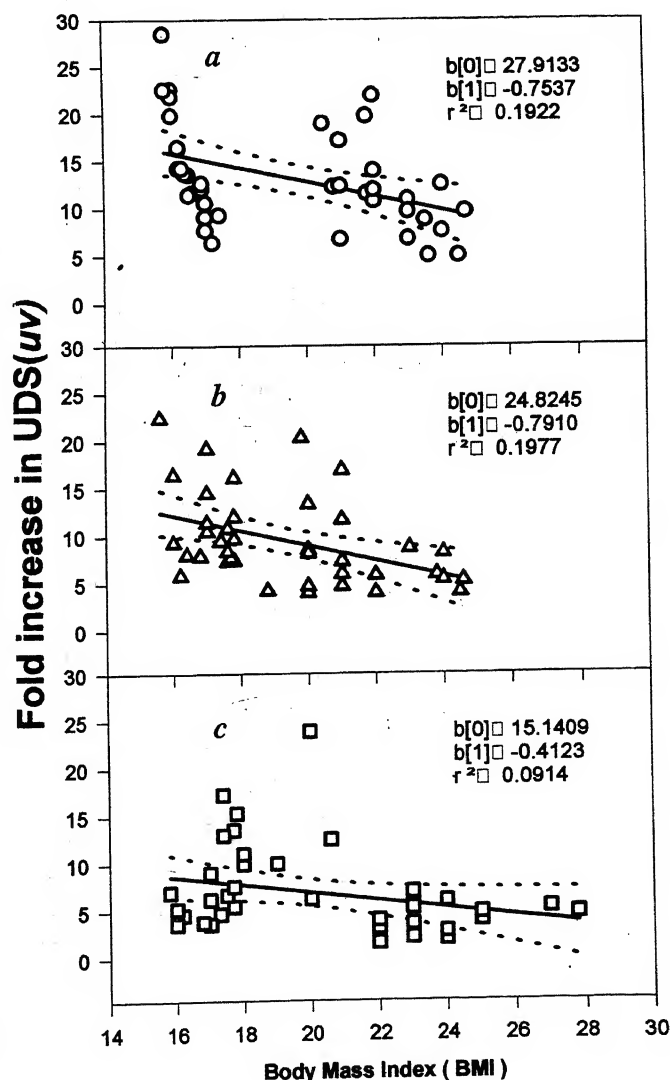


Figure 2. Ultraviolet light (UV) induced fold increase in DNA repair in the peripheral lymphocytes of human subjects as a function of Body Mass Index at young (a), adult (b) and old (c) ages. Linear regression analyses are shown. Other details are the same as in Figure 1.

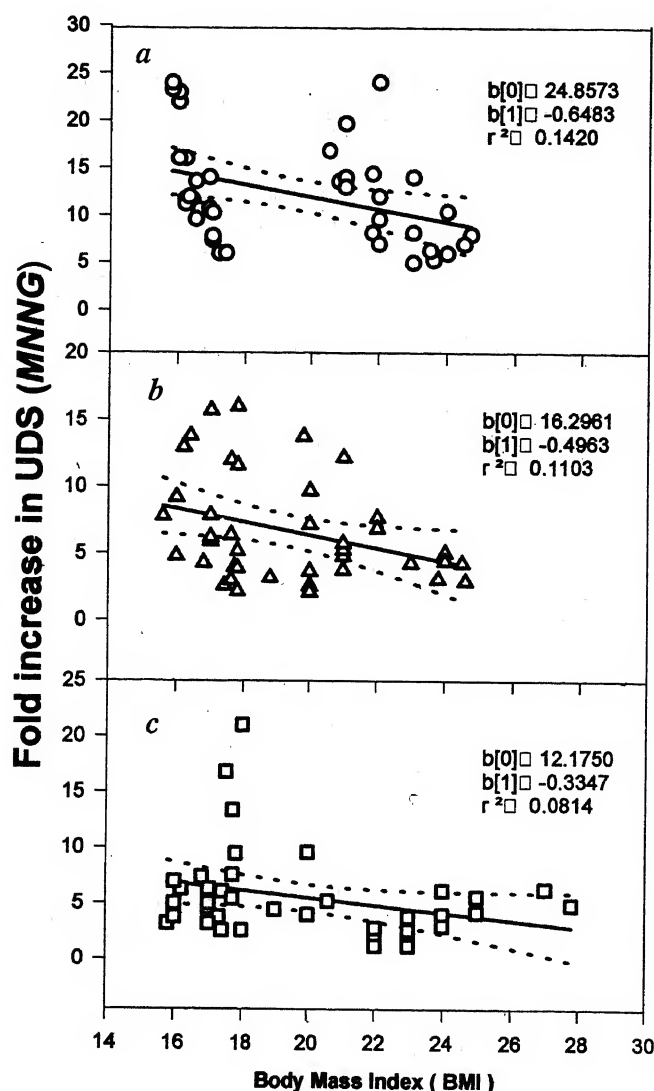


Figure 3. MNNG-induced fold increase in DNA repair in peripheral lymphocytes of human subjects as a function of Body Mass Index at young (a), adult (b) and old (c) ages. Linear regression analyses are shown. Other details are the same as Figure 1.

The average BMI of the subjects in the various age groups are shown in Table 1 a. Since the subjects were selected after careful examination of their familial and dietary history (most of these subjects belong to lower to upper middle class society and largely belong to either this university community or old age homes with a steady income behind them) and extensive clinical examination (data shown in Table 1 b), the low BMI value is taken to indicate a chronic but natural intake of low calories. Thus this LBMI group is assumed to be similar to an experimentally diet-restricted animal and therefore considered as 'undernourished' without any apparent malnutrition. Lymphocytes from these subjects were examined for various DNA repair parameters under different conditions.

Table 2. Basal and induced UDS in peripheral lymphocytes of human subjects with normal BMI (normal) and low BMI (undernourished) at different ages

Status	Basal repair	Induced repair	
		UV	MNNG
<i>Young:</i>			
Normal BMI	7.3 ± 3.7	83.4 ± 36.2	70.6 ± 33.6
Low BMI	9.2 ± 4.3	123.2 ± 49.9*	106.7 ± 46.0*
<i>Adult:</i>			
Normal BMI	6.5 ± 4.5	43.0 ± 29.9	35.3 ± 22.6
Low BMI	11.2 ± 5.8*	117.4 ± 50.1*	110.6 ± 62.0*
<i>Old:</i>			
Normal BMI	5.3 ± 4.5 [†]	27.6 ± 27.2	21.4 ± 17.6
Low BMI	11.4 ± 10.7*	87.3 ± 78.8*	78.6 ± 76.0*

Values are expressed as femtomoles of [^3H]-thymidine incorporated per μg DNA and represent average from 20 individual experiments. Unscheduled DNA synthesis (UDS) is measured as follows: Lymphocytes at a concentration of $10^6/\text{ml}$ in RPMI-1640 medium with 2 mM glutamine, 100 IU/l penicillin, 100 $\mu\text{g}/\text{l}$ streptomycin and 10% fetal calf serum, were incubated with and without 5 mM hydroxyurea (HU) and 5 $\mu\text{Ci}/\text{ml}$ [^3H]-thymidine (specific activity - 17Ci/mmol) for 2 h in a CO_2 incubator at 37°C with 90% humidity. The radioactivity incorporated into DNA was measured by standard procedure⁴⁴. The radioactivity in the cells incubated with HU is taken as the measure of UDS (DNA repair). *These values are significantly different from those of corresponding age matched NBMI group at a p value of ≤ 0.05 . † This value is significantly different from that of NBMI group of 'young' age at a P value of ≤ 0.05 .

Lymphocytes from anticoagulated blood of the subjects in the above groups were isolated by Ficoll-paque density gradient centrifugation^{24,25} and the unscheduled DNA synthesis (UDS), which is a measure of DNA repair, was examined in the peripheral lymphocytes. For the statistical analysis the Student's t test (paired) was used and the results are shown in Table 2. The basal DNA repair in NBMI subjects is decreased by 27% in old age as compared to 'young'. The difference among the young and adult groups was found to be not significant. In the LBMI group, however, no significant difference was noticed in the basal repair capacity among the three age groups and also the values at adult and old ages were higher than the corresponding age matched normals ($p \leq 0.05$). When the lymphocytes were challenged with a mutagenic treatment of exposure to either ultraviolet (UV 254 nm, $20 \text{ J}/\text{m}^2$) irradiation or to 50 μM of *N*-methyl-*N'*-nitro-*N*-nitroso-guanidine (MNNG), there was a response in the groups by way of increased UDS. The increased UDS due to this mutagenic treatment is also shown in Table 2. In normal individuals, there is a decrease in this response with age. However in the case of LBMI individuals, the response was always higher as compared to the normal group and this trend was similar in all the three age groups studied. This is to be expected because the LBMI groups already

had a higher level of basal repair and when this is coupled with the better response to mutagenic challenge, the result is a distinctly improved DNA repair capacity in LBMI subjects.

The DNA repair capacity as revealed by UDS is also examined as a function of BMI in each of the age groups by generating computerized regression curves (Sigma plot program, version 2.01), where BMI values are plotted against DNA repair. Figure 1 *a*, *b* and *c* show the regression analyses for 'young', 'adult' and 'old' age groups along with 95% confidence limits. As can be seen, BMI, therefore the nutritional status, has practically no correlation with DNA repair level in young, weak correlation in adult, but significant correlation in old. In line with this, the differences between the average values of low BMI group and normal BMI group at different ages were statistically significant at $P \leq 0.05$ level (Table 2). Similar regression analysis curves are shown for the UV (Figure 2) and MNNG (Figure 3) induced fold increase in DNA repair as a function of BMI at all the three ages. It can be seen that there is always an inverse correlation, albeit to varying degrees, depending upon the age and the mutagen employed, between the BMI and the fold increase in DNA repair. In all these analyses, only the basal DNA repair in 'young' has failed to show any relation with BMI.

These results with human subjects are in good agreement with the findings of earlier workers on experimental animals when dietary restriction was shown to improve the unscheduled DNA synthesis in lymphocytes, hepatocytes and kidney cells in rats²⁶⁻²⁸.

DNA repair in mammalian cells is a complex process involving many gene products²⁹. The overall process consists of recognition of the damage, excision of the damaged portion, resynthesis of the excised portion and finally ligation of the last nucleotide gap. Several endonucleases/protein factors were identified for the recognition of the damage and incision at the damaged site²⁹. During the past several years, we have identified two major endodeoxyribonucleases, one with an acidic pH optimum and the other with alkaline pH optimum, in rat brain. Detailed studies on the properties of these enzymes suggested a role in DNA repair at the initial incision step. The enzyme with acidic pH optimum was able to attack UV irradiated DNA while the second enzyme with alkaline pH optimum could attack a variety of damaged DNAs including apurinic/apyrimidinic DNA (AP-DNA)^{30,31}. An endonuclease acting on UV irradiated DNA and an AP endonuclease have been shown in mammalian tissues/cells including brain^{32,33}. Similarly DNA-polymerase β , generally considered to be a repair enzyme especially in base excision repair and also shown to be induced against damage to cellular DNA^{34,35} is found to be the major polymerase in adult brain^{36,37}. We have recently shown that the activity of this enzyme decreases in the aging rat brain possibly due

Table 3. Basal (BA) and UV induced (IA) DNA-polymerase activity in peripheral lymphocytes of subjects with normal BMI and low BMI at different ages

Status	DNA polymerase activity	
	BA	IA
<i>Young:</i>		
Normal BMI	395 \pm 167	732 \pm 350
Low BMI	424 \pm 158	911 \pm 386
<i>Adult:</i>		
Normal BMI	204 \pm 51	361 \pm 109
Low BMI	249 \pm 63*	421 \pm 113
<i>Old:</i>		
Normal BMI	136 \pm 69	240 \pm 160
Low BMI	204 \pm 69*	362 \pm 171*

Values are expressed as picomoles [³H]-TMP incorporated per mg of protein per hour. DNA polymerase assay: The preparation of cell extracts and assay was carried out according to the procedure of Nagasaka and Yoshida⁴⁵, which is optimally suited for measuring the DNA-polymerase β activity. Other details are as in Table 2. *These values are significantly higher than those of corresponding NBMI group at a P value of ≤ 0.05 .

to post-translational modification of the enzyme molecules³⁸. In view of this information, we have measured the activities of DNA polymerase β , UV DNase and AP DNase as markers of DNA repair potential in the lymphocytes of the subjects under study.

The activities of DNA polymerase β in lymphocytes with and without UV challenge are shown in Table 3. There is a decrease in the basal activities in both the normal and LBMI subjects, as age progresses, but the enzyme levels are significantly higher in the LBMI when compared to normals in adult and old age groups. Even under induced conditions, the pattern remained the same in that while the age dependent decrease is there in both groups, the values were always higher in LBMI group. However, statistical analysis showed that only in the old age the differences between NBMI and LBMI groups were significant. It can be seen that at old age the values in LBMI group are 50% higher than those in NBMI group. The activity profile observed in cells stimulated with phytohemagglutinin (PHA) and with and without UV challenge was also seen to follow a similar pattern (data not shown). The observed increase in basal DNA repair (Table 2) in the LBMI group at adult and old ages may be attributed to these enhanced activities of β polymerase. It is interesting that Srivastava and Busbee³⁹ have shown that both α and β polymerases of calorie restricted aged mice exhibit a higher level of fidelity than polymerases of *ad libitum* fed aged mice.

The activities of two DNases, UV DNase and AP DNase, in the lymphocytes of the subjects are shown in Table 4. An age associated decline in basal levels of UV DNase is seen among the normal subjects. The LBMI

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Table 4. Basal (BA) and UV induced (IA) activities of UV DNase and AP DNase in peripheral lymphocytes of subjects with normal BMI and low BMI at different ages

Status	UV DNase activity/mg DNA		AP DNase activity/mg DNA	
	BA	IA	BA	IA
<i>Young:</i>				
Normal BMI	199 ± 37	341 ± 64	246 ± 70	363 ± 70
Low BMI	210 ± 45	368 ± 56	287 ± 66	375 ± 54
<i>Adult:</i>				
Normal BMI	140 ± 35	236 ± 66	208 ± 39	355 ± 115
Low BMI	258 ± 123*	363 ± 159*	253 ± 117	365 ± 167
<i>Old:</i>				
Normal BMI	126 ± 57	160 ± 71	133 ± 65	176 ± 102
Low BMI	222 ± 68*	260 ± 64*	237 ± 55*	276 ± 72*

Values are expressed as μg of DNA-P liberated per mg DNA and are the averages of 20 individual experiments. DNases assay: UV and AP DNases were assayed essentially according to the procedure of Rao and Rao⁴⁶. Substrate for UV DNase: UV DNA prepared by UV irradiation of highly polymerized calf thymus DNA (2 mg/ml) at a dose of $2 \times 10^4 \text{ J/m}^2$ using Phillips TUV 8 15 W germicidal lamp. Substrate for AP DNase: Depurinated DNA prepared essentially as described earlier⁴⁷. Native calf thymus DNA (2 mg/ml) mixed with equal volume of depurination buffer (40 mM sodium citrate, 40 mM NaCl, 40 mM potassium phosphate, pH 5.0 incubated at 70°C for 15 min). Other details are as in Table 2.

*These values are significantly higher than those of the corresponding NBMI group at a P value of ≤ 0.05 .

group at young age showed marginally higher values which were not statistically significant. However, at both adult and old ages, the values were significantly higher in LBMI group as compared to normal. Also the age-dependent decline seen in normal individuals is not evident in the LBMI individuals. A similar pattern of changes is seen even after the lymphocytes are exposed to UV except that the values are higher.

In the case of AP DNase, the pattern of changes in basal activities is similar to UV DNase. UV exposure of the lymphocytes resulted in comparable induction of this activity in both normal and LBMI groups at all ages, but in old age both the basal as well as induced activities in LBMI groups were significantly higher than those in NBMI group. Thus the overall picture seems to be that either the activities are unaffected or higher in LBMI individuals.

Age-related decline in UV-induced DNA repair has been reported in human peripheral lymphocytes^{40,41} and also in tissue from the central nervous system⁴². The present results while confirming the age-dependent decline in the activities of DNA repair enzymes also show that human subjects with low BMI presumably facing chronic low calorie consumption exhibit either unaffected or even improved activities with the age-dependent decline being distinctly slower.

This is the first study of its kind on human subjects to demonstrate the beneficial effects of chronic undernutrition, a phenomenon reported earlier in experimental

animals²⁶⁻²⁸. Walford and his group are conducting an elaborate experiment to examine the effects of diet on general physiology of humans including aging⁴³. These experiments are still continuing. However the present study is planned and executed with a population that is naturally available in India. The results indicate the possibility of a very intriguing and interesting phenomenon that LBMI (moderate undernutrition with no apparent malnutrition) is actually beneficial in human subjects in maintaining good DNA repair capacity. How undernutrition is able to achieve this is a matter of speculation. It is logical to expect that in LBMI individuals the cellular metabolism would be at a low ebb. However, even against this reduced calorie availability, the cell might choose to maintain certain essential pathways at normal rates and DNA repair pathway could be one such pathway in view of its cardinal role in protecting the genomic integrity. This in itself may be a major contributing factor for improved longevity in such human subjects.

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A simple technique to expose tree seedlings to elevated CO₂ for increased initial growth rates

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Initial growth rates of most tree species that are used in afforestation programmes are very low. Therefore, polybag planted seedlings have to be maintained in the nurseries for a long period of time. Growing plants in an elevated CO₂ atmosphere increases the growth rates as well as biomass production in many annual crop and tree species. Higher temperature and relative humidity in association with elevated CO₂ concentration helps to boost the biomass and leaf area production. We demonstrate here an easy and cost-effective method for obtaining elevated CO₂ concentrations for better growth of tree seedlings in the nursery.

APART from maintaining a balanced ecosystem, forests are major sinks of CO₂. Deforestation and burning of fossil fuel (due to population pressure) are the two major reasons for accumulation of CO₂ in the atmosphere leading to global climate change. Currently the CO₂ concentration in the atmosphere is around 360 ppm and it is increasing at the rate of 1.8 ppm per year¹. Hence afforestation is a practically feasible way to address the global climate change, especially in tropical countries where forest felling is occurring at a faster rate.

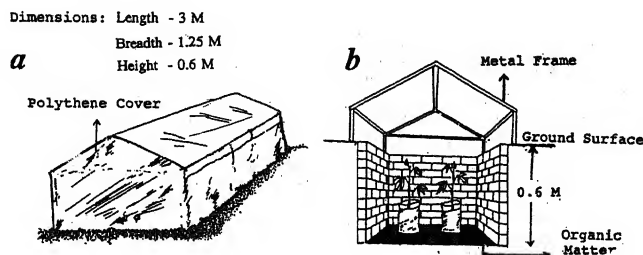


Figure 1a, b. Set up of the CO₂ enrichment system used for growing seedlings under elevated CO₂ concentration. a, Depicts the polythene cover enclosing the seedlings inside the trench; b, Cross-sectional view of the trench in which seedlings are kept and enclosed to expose to elevated CO₂ concentration. Floor of the trench is spread with decomposed organic matter. A metal frame is placed on the trench that supports the polythene cover to enclose the seedlings.

For any successful afforestation programme it is necessary to produce robust and healthy seedlings. Growing plants under elevated CO₂ concentrations has proved to enhance the biomass production². For obtaining elevated CO₂ concentrations, there are different systems available. Depending upon the need and purpose, one can select the desirable type of CO₂ enrichment system. These include, closed systems in controlled environment cabinets, 'Solardome' glass house, open top chambers, FACE (free air CO₂ enrichment), etc³.

There are advantages and disadvantages associated with these methods. However, one major constraint in most of these techniques is the high cost involved to expose the plants to elevated CO₂. In the present study we have developed a simple and economic technique to grow polybag seedlings under elevated CO₂ concentrations obtained from decomposition of organic matter. We demonstrate its successful use in obtaining increased growth rates in thirteen tree species at seedling stage.

Rectangular trenches of 3 m length, 1.25 m width and 0.6 m depth, were made in a place exposed to open sunlight. All the four sides of the cut ends of the trench were provided with a single layer of brick lining to avoid sliding of the edges. Inside the trench a layer of well decomposed organic matter was spread uniformly all along the floor. 16 kg of organic matter was required for the trench of above dimension to get a CO₂ concentration of 700–750 ppm (Figure 1).

A rectangular metal frame was placed over the trench completely enclosing it. The frame was fabricated using hollow galvanized iron tube of 1.5 cm diameter. Height of the frame was 1.6 m with a gable roof. Using high density polythene sheet of 125 μ gauge, a cover was tailored to suit the size of the frame. Length of the polythene cover was 12 inches longer than the frame so as to allow a free and flat fall on the ground after enclosing the metal frame. On this free lying polythene, a thin layer of sand was poured to keep the complete system air tight.

In this system we made use of the CO₂ produced by decomposition of organic matter spread on the floor of the trench. CO₂ thus released was trapped in the polythene chamber and a concentration of 700–750 ppm was obtained. Seedlings were exposed to higher concentrations of CO₂. Polybag seedlings were arranged in the trench so that the leaves of adjacent plants did not overlap and cause mutual shading (Figure 1b). Seedlings were exposed to elevated CO₂ between 3.30 pm and 11.00 am. Before closing the trenches water was sprinkled on the organic matter to stimulate soil respiration. Polybag seedlings were also watered before closing the chamber. In this system, apart from CO₂, relative humidity and temperature also built up inside the chamber.

Photosynthetic photon flux (PPF) over a wavelength of 400–700 nm, CO₂ concentration and relative humidity were measured using LI-6000 Portable Photosynthesis system (LI-Cor Inc, Nebraska, USA). PPF measurement inside the trench was made at 15 cm below the roof of the polythene structure. Temperature inside the trench and ambient air were measured simultaneously. Leaf area was measured using portable leaf area meter (LI-3000, LI-Cor Inc, Nebraska, USA). The thirteen species studied were – *Annona squamosa*, *Zizyphus jujuba*, *Tamarindus indica*, *Acacia auriculiformis*, *Derris indica*, *Spathodia campanulata*, *Feronia elephantum*, *Artocarpus integrifolia*, *Swietenia microphylla*, *Eucalyptus citriodora*, *Tectona grandis*, *Dalbergia latifolia* and *Dalbergia sissoo*.

Carbon dioxide concentration inside the trench started to build after enclosing the trenches at 350 ppm and reached 700–750 ppm in less than an hour. It remained high all through the night and started decreasing after sunrise in the morning (Figure 2). There was no difference in the concentration of CO₂ thirty minutes after closing the trenches in the air samples drawn from different heights inside the trench. In the morning, photosynthetically active radiation started increasing (Figure 3a) corresponding with the decrease in the CO₂ concentration inside the trench (Figure 2b), suggesting the utilization of higher concentration of CO₂ by the plants in the presence of sunlight for photosynthesis. Simultaneously, rise in the temperature inside the trenches was also more after sunrise (Figure 3b). An average of 1.21°C rise was seen inside the chamber compared to ambient air up to 8.00 am, while this difference from 8.00 am to 11.00 am was 4.69°C. Therefore, it was not possible to expose the seedlings for long periods after 11.00 am. Along with CO₂ and temperature, build up of relative humidity was also noticed inside the trench (Figure 3c). RH built up inside the chamber within thirty minutes after closing and reached the saturation level. It remained so till the trenches were exposed to open air, next day.

Using this system, seedlings of thirteen tree species were exposed to elevated CO₂, relative humidity and

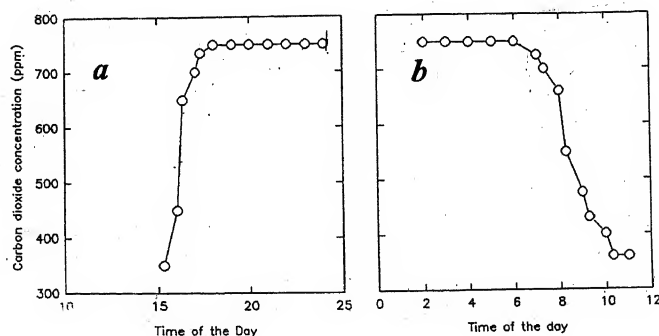


Figure 2a, b. Changes in the CO₂ concentration inside the chamber. a, Build-up of the CO₂ concentration inside after enclosing the trench (1530 h to 0600 h). b, Depletion of CO₂ concentration after 0700 h up to 1100 h.

Table 1. Dry matter (g/plant) production after ninety days of growth of seedlings under ambient air and elevated CO₂ concentrations

Species	Dry matter produced per plant (g/plant)		Increase in dry matter production in elevated CO ₂ plants over ambient air grown plants (%)
	Grown under ambient air	Grown under elevated CO ₂	
<i>Annona squamosa</i>	6.14	8.32	35.5
<i>Zizyphus jujuba</i>	4.24	9.54	125.0
<i>Tamarindus indica</i>	3.96	4.45	12.3
<i>Acacia auriculiformis</i>	9.30	13.60	44.0
<i>Dalbergia sissoo</i>	4.40	12.20	193.0
<i>Spathodia campanulata</i>	7.90	14.80	88.0
<i>Derris indica</i>	4.04	9.50	135.0
<i>Feronia elephantum</i>	4.75	5.58	17.0
<i>Artocarpus integrifolia</i>	3.40	8.70	155.0
<i>Swietenia microphylla</i>	7.88	11.03	101.0
<i>Eucalyptus citriodora</i>	5.54	8.38	51.0
<i>Tectona grandis</i>	0.70	3.92	460.0
<i>Dalbergia latifolia</i>	6.14	8.38	36.0
Mean	5.26	9.10	111
C.D. at 5%			
Treatment 0.60			
Species 0.63			

temperature. Six-month-old seedlings were subjected to this treatment for ninety days and their responses were assessed to study the effectiveness of the system described.

There was a positive response to elevated CO₂ in biomass production, though the extent of response varied with the species. From this data it is evident that growth rates were better under elevated CO₂ concentration (Table 1). Among the various species, *Tectona grandis* showed the highest response to elevated CO₂ followed by *Dalbergia sissoo* and *Artocarpus integrifolia* in terms of biomass production. The total dry matter of *T. grandis* was 460 per cent more under elevated CO₂, compared to that of ambient air grown seedlings.

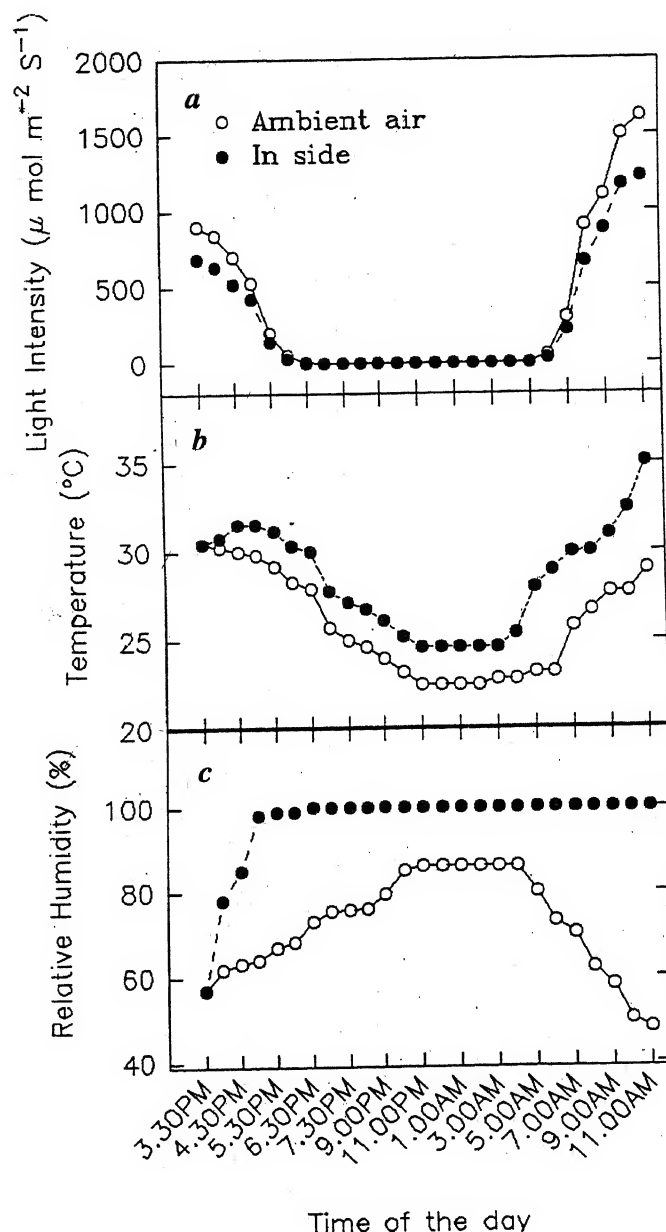


Figure 3a-c. Changes in (a) light intensity, (b) temperature and (c) relative humidity in the ambient air (O) and inside the chamber (●) for the period during which the seedlings were exposed to elevated CO₂ concentration.

The least responsive was *Tamarindus indica* recording only 12% increase (Table 1).

In this system, plants are making use of higher concentrations of CO₂ for carbon assimilation in the presence of light in the morning hours between 7.00 and 11.00 am. Evidences from a large body of literature show that it is possible to increase the biomass production by growing plants under elevated CO₂ (refs 2, 4, 5). In the present method of CO₂ fertilization, plants were

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Table 2. Leaf area (cm) produced after ninety days growth under elevated CO₂ and ambient air grown seedlings

Species	Leaf area per plant (cm ²)		Increase in leaf area of elevated CO ₂ plants over ambient air grown plants (%)
	Grown under ambient air	Grown under elevated CO ₂	
<i>Annona squamosa</i>	45	189	320
<i>Zizyphus jujuba</i>	73	260	256
<i>Tamarindus indica</i>	61	78	27
<i>Acacia auriculiformis</i>	224	415	85
<i>Dalbergia sissoo</i>	173	641	270
<i>Spathodia campanulata</i>	260	824	216
<i>Derris indica</i>	155	520	235
<i>Feronia elephantum</i>	113	164	45
<i>Artocarpus integrifolia</i>	74	326	340
<i>Swietenia microphylla</i>	278	426	53
<i>Eucalyptus citriodora</i>	334	454	35
<i>Tectona grandis</i>	32	356	1012
<i>Dalbergia latifolia</i>	115	197	71
Mean	149	373	228
C.D. at 5%			
Treatment 20.12			
Species 21.21			

also experiencing relatively higher temperatures compared to ambient air grown plants. Imai and Murata⁶, noticed enhancement of leaf area and whole plant dry weight at higher CO₂ associated with higher temperature. Long⁷ has shown that proportionate increase in photosynthesis resulting from elevated CO₂ will rise with temperature. He also suggested that elevated CO₂ alters the magnitude of response of leaf canopy carbon gain to rising temperature and relative humidity which apparently enhances growth. It is likely that temperature and humidity associated with higher concentration of CO₂ must have helped in higher carbon assimilation and leaf expansion rates respectively.

Another important plant character that showed a remarkable increase under elevated CO₂ was leaf area per plant. A significant increase in leaf area was noticed among the species under elevated CO₂ when compared to ambient air grown plants (Table 2). All the species responded positively to elevated CO₂ concentration. An average of 228% increase in leaf area production was recorded under elevated CO₂ (mean of all the thirteen species studied). Similar type of studies conducted under controlled conditions have reported increase in leaf area in plants grown under high concentrations of CO₂ (refs 8–10). When higher temperature is associated with elevated CO₂ concentrations, leaf area is further enhanced¹¹. It is due to higher leaf initiation rate, leaf expansion and individuals leaf area. Saman *et al.*¹² have shown that leaf elongation rates were more under elevated CO₂ concentrations and were associated with

higher sucrose phosphate synthase activity during the early vegetative stage when growing blades were strong carbohydrate sinks. Leaf elongation rate is one of the ways by which leaf area development can be stimulated. Relative humidity is another important weather parameter that determines leaf growth. Elevated CO₂ concentrations, associated with higher temperature and RH might have influenced the leaf expansion rates. Relative humidity can reduce the vapour pressure deficits and thus help in maintaining high turgidity of the cells providing congenial conditions for cell division, elongation and expansion. Another possible reason for higher growth seen under this system could be due to lower dark respiration rates. According to Tanaka¹³, efficiency of respiration increases as a result of whole plant CO₂ enrichment. Decreased rates of respiration at higher concentrations of CO₂ was noticed^{14,15}. Even a small change in the respiration rate can have a considerable influence on assimilate retention and therefore its utilization for growth processes.

In the above method described for growing polybag seedlings of forest tree species, the main advantage is that there is no need of adding CO₂ from an external source, thereby reducing total cost considerably. Construction of the system is within the scope of a small workshop and material required is easily available. This system can be effectively used to enhance the initial growth rates of the seedlings specially forest tree species which have very poor growth rates. This can help in getting robust seedlings that may perform better when field planted and also helps in reducing the casualty.

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Studies on molluscan diversity in Kaveri river system (Tiruchirappalli, India) with special reference to vector snails of trematode parasites

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A monthly collection of molluscan animals was carried out from 11 sampling stations (K1-K11) of Kaveri river stretch from Karur to Grand Anicut (95 km) for a period of two years (April 1991–March 1993). 13 species of molluscs were recorded, of which 8 species were gastropods and 5 species bivalves. The gastropod species were grouped under 5 different families (Viviparidae, Thiaridae, Pilidae, Lymnaeidae and Planorbidae) and the family Thiaridae was the most dominant group representing 50% of the total gastropod population. Five species of bivalve molluscs were classed under 2 different families namely Unionidae and Corbiculidae. The mean density of molluscs was higher ($96/m^2$) in sector I (K1–K4) than in sector II ($39/m^2$) which include stations K5–K11. Five gastropod species were identified as carriers of the disease causing parasite cercarial larvae. The pulmonate snails were preferred most by trematode cercaria.

SEVERAL investigations were undertaken on major benthic animal groups of freshwater system. Notable contri-

butions to our knowledge of molluscan fauna have been made by several authors¹⁻⁵. Hoagland and Brown^{6,7} made an extensive investigation exclusively on gastropods. Relationship between gastropod density and vegetation has been reported by many authors⁸⁻¹⁰. Similarly bivalve species density has been studied by several workers¹¹⁻¹³. Srinivasan¹⁴ has reported three species of freshwater mussels in the Kaveri river system, namely *Lamellidens marginalis* (Lamarck), *L. conso-brinus* (Lea) and *Parreysia favidens* (Benson). However, an intensive study on molluscan fauna of the Kaveri river has not been made in recent times.

Freshwater snails are preferred intermediate hosts for cercaria larvae of trematode parasites¹³. A number of trematodes of horse, goat, sheep, camel, dog, buffalo and other cattle develop to cercarial stage in certain gastropod molluscs. They cause diseases and mortality in cattle. About 57 types of cercariae were recorded from the snail *Indoplanorbis exustus*⁵. The distribution of this snail was reported throughout the plains of India, Pakistan, Persia, Srilanka, Burma, Malaya, Indochina, Thailand, Sumatra, Java and Celebes⁵. Burch¹³ has also reported cercarial larvae in the two distinctly different freshwater snails in the Eastern African regions. Since these parasites caused considerable damage to the cattle wealth of the river basin¹⁵, the parasitology of these trematode worms assumed economic importance. Studies on the snails acting as vectors of disease causing trematode parasites in the Kaveri river are very limited¹⁶. Hence, an attempt has been made to identify the snail vectors of trematode parasites inhabiting the Kaveri river.

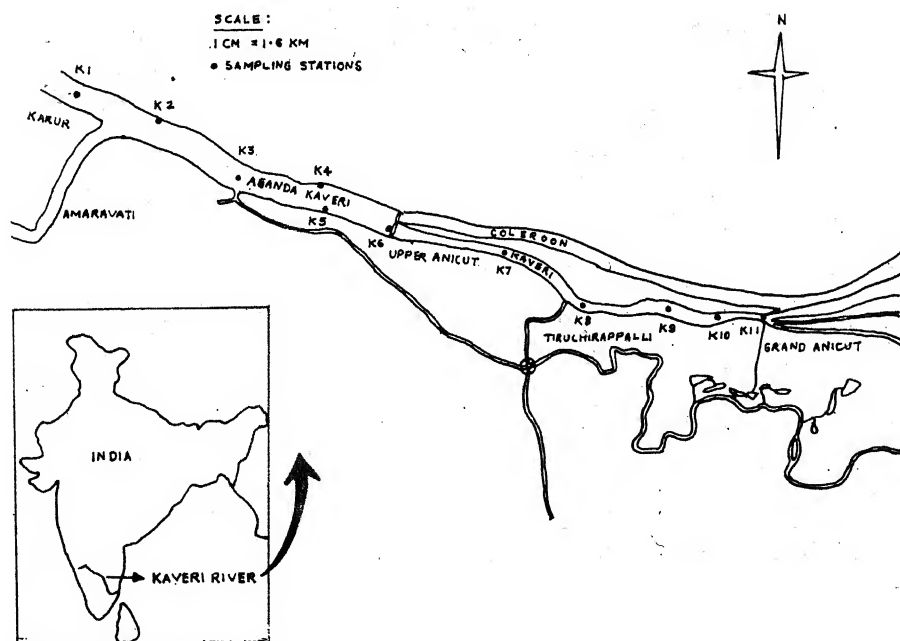


Figure 1. Location of the Kaveri river and sampling sites.

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A study on the molluscan fauna of the Kaveri river stretching from Karur to Grand Anicut at Tiruchirappalli (10°48'N and 78°42'E) was carried out for a period of 2 years from April 1991 to March 1993. The river stretch under study is a fifth order stream and broadest after receiving several tributaries. Eleven sampling stations (K1-K11) were fixed in the river stretch (Figure 1). The river stretch under study is divided into two sectors based on the degree of human interference, i.e. sector I (K1-K4) and sector II (K5-K11). The urban agglomeration of Tiruchirappalli is centred around sector II and hence, it is found to have higher human interference than sector I.

The molluscan animals were collected from 11 sampling stations of the Kaveri river system. Larger molluscan species were collected by hand picking and also sieving the river sediments by using coarse sieves. The bottom substrate and macro-vegetations were explored for collecting the most representative diversity of molluscs and they were identified. Snails associated with vegetation were collected by using hand net (150 µm). The hand net was dragged over the aquatic vegetation. When the net was filled with aquatic weeds, the contents were poured out on a spread out cloth piece. The population density of molluscs in a given area was studied using quadrat method as described by Subbarao⁵. The number of animals in the samples collected from wooden quadrant (0.5 × 0.5 m) was counted and value expressed as the number of molluscs per square meter (number/m²).

Snails were collected manually, using gloves to prevent cercarial infection. The collected snails were then transported to the laboratory in polythene bags along with weeds and water. Then the live snails were individually transferred to clean glass test tubes half filled with tap water and exposed to sunlight for discharge of cercaria from the snails¹⁷. The cercaria were transferred onto glass slides with fine pipette and observed under microscope. Some of the cercariae were fixed in 10% formalin stained with eosin or methylene blue mounted in DPX, and identified.

The present study revealed that the molluscan fauna of the Kaveri river were represented by 8 species of gastropod and 5 species of bivalves. The gastropod species are classed under 5 different families, viz. Viviparidae, Thiariidae, Pilidae, Lymnaeidae and Planorbidae; the family Thiariidae being more dominant and representing nearly 50% of the total gastropod population recorded in the Kaveri river (Figure 2). The identification of species was confirmed by the Zoological Survey of India, Calcutta. Certain species of gastropods such as *Bellamya dissimilis*, *Paludomus tanschaureicus*, *Thiara* sp., were found predominant in the Kaveri river throughout the year. Gastropod species such as *I. exustus*, *Lymnaea ovalis*, *P. virens* and *B. dissimilis* were associated with aquatic macrophytes.

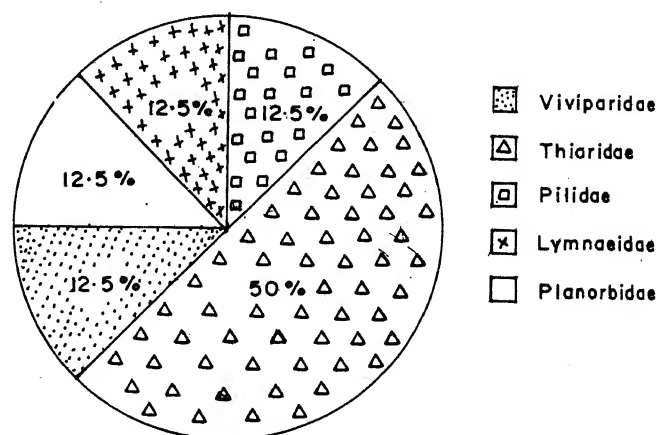


Figure 2. Percentage of species composition of gastropod molluscs in Kaveri river stretch from Karur to Grand Anicut.

Table 1. Classification of molluscan fauna in Kaveri river stretch in Tiruchirappalli

Phylum	:	Mollusca
Class	:	Gastropoda
Subclass	:	Prosobranchia
Order	:	Mesogastropoda
Family	:	Viviparidae
Genus	:	<i>Bellamya</i>
		<i>B. dissimilis</i>
Family	:	Thiariidae
Genus	:	<i>Thiara</i>
		<i>T. (Thiara) scabra</i>
		<i>T. (Tarebia) lineata</i>
		<i>Paludomus tanschaureicus</i>
Family	:	Pilidae
Genus	:	<i>Pila</i>
		<i>P. virens</i>
Subclass	:	Pulmonata
Order	:	Linophila
Family	:	Lymnaeidae
Genus	:	<i>Lymnaea</i>
		<i>L. ovalis</i> Gray
Family	:	Planorbidae
		<i>Indoplanorbis exustus</i> (Deshayes)
Class	:	Bivalvia (Linnaeus, 1758)
		Pelecypoda (Goldfuss, 1820)
Subclass	:	Lamellibranchia
Order	:	Eulamellibranchia
Family	:	Unionidae
Genus	:	<i>Lamellidens</i> (Lamarck)
		<i>L. consobrinus</i> (Lea)
		<i>L. marginalis</i> (Lamarck)
Genus	:	<i>Parreysia</i>
		<i>Parreysia favidens</i> (Benson)
Family	:	Corbiculidae
Genus	:	<i>Corbicula</i>
		<i>C. regularis</i>
		<i>C. striatella</i>

Table 2. Seasonal variation in the molluscan faunal density in two sectors of the Kaveri river

Seasons	Sector I (no./m ²)	Sector II (no./m ²)
Premonsoon (April–July)		
1991–1992	242 ± 18	67 ± 14
1992–1993	88 ± 14	25 ± 8
Mean	126 ± 28	45 ± 23
Range	72 – 267	20 – 98
Monsoon (August–November)		
1991–1992	44 ± 12	23 ± 13
1992–1993	60 ± 17	25 ± 16
Mean	53 ± 13	23 ± 18
Range	24 – 92	14 – 42
Postmonsoon (December–March)		
1991–1992	139 ± 15	44 ± 13
1992–1993	77 ± 14	23 ± 8
Mean	110 ± 19	33 ± 15
Range	68 – 157	16 – 68
I year mean	115 ± 23	45 ± 12
II year mean	76 ± 24	33 ± 13
Annual mean	96 ± 29	39 ± 18
Range	24 – 267	14 – 117

Five species of bivalve molluscs belonging to 2 different families such as Unionidae and Corbiculidae were recorded (Table 1). The abundance of molluscan species was quantified at two sectors of the Kaveri river. The mean density of molluscs at sector I was 96/m² and it ranged from 24 to 267/m² and in sector II, the mean density of molluscs was 39/m² and it ranged from 14 to 117/m² (Table 2). The abundance of molluscs was significantly different between the sectors (ANOVA: $F = 21.45$; $P < 1\%$) whereas it was not significantly different within the sector (ANOVA: $F = 1.96$; $df = 23$; $P > 1\%$).

Eight species of gastropods recorded in the present study were tested for the presence of cercaria larvae. Five species namely, *Indoplanorbis exustus*, *Lymnae ovalis*, *Thiara tuberculata*, *T. scabra* and *Paludomus tanschauricus* were carriers of the cercarial larvae. Cercarial infection was more acute in *I. exustus* and *L. ovalis* than in other snail vectors of the Kaveri river. Since these parasites caused considerable damage to the cattle wealth of the Kaveri river basin, the parasitology of these trematode worms assumed economic importance. Identification of such intermediate snail host and effective control of snail population would be the best preventive measure¹⁵.

The present study also indicated that species of the Thiariidae such as *T. scabra* and *T. lineata* were found to prefer the pollution-free water and sandy bottom, which provide soft substratum for their easy penetration.

Therefore, they were predominantly recorded in sector I than sector II. Among the gastropod species, *I. exustus*, *L. ovalis*, *P. virens* and *B. dissimilis* were associated with aquatic weeds which provided food and shelter for those organisms. A similar study on correlation between aquatic weeds and molluscan abundance was made by Upatham¹⁸ in Thailand. Anwar and Siddiqui¹⁹ recorded 7 species of gastropods and 3 species of pelecypods in Kali river.

Molluscan groups such as gastropods and bivalves were found to be quantitatively most important groups and represented throughout the year^{20,21}. Kaul *et al.*⁹ reported that distribution of freshwater gastropods in Haigam, a typical wetland in Kashmir, had bearing on density of aquatic weeds.

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Evidence of Archaean crustal shortening from deformed pillow lavas: An example from Sandur greenstone belt, Dharwar craton

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There is a continuous debate about the autochthonous or allochthonous model of the evolution of the greenstone belts. We report here evidence which suggests that the Sandur greenstone belt of Dharwar craton is an allochthonous remnant of accreted oceanic volcanism. Pillow structures of the western margin of the Sandur Schist Belt have been compressed in NE-SW and stretched in N 40°-45°W direction. The length/width (L/W) ratio (X'/Y') of these elongated pillows varies from 3 to 30, transforming them to pencil shape with well developed schistosity. Poisson's ratio of the elongated pillows at uniaxial stress of 1.5 kb is 1:24, whereas that of undeformed pillows 1:10 or 1:14. Such compression has not been witnessed in the eastern part of the belt. Generally, pillows in Sandur and other greenstone belts in an undeformed state show length/width ratio varying between 1 and 2. The comparison of the L/W ratio of the elongated and normal pillows and their Poisson's ratios from the western and eastern parts show that 7 to 8 times shortening has taken place along the western margin of the belt. This shortening along the belt is interpreted due to horizontal compression, consequent of convergent margin movement and rock mass transport, implying their allochthonous character.

It has been frequently suggested that a modified form of plate tectonics (smaller plates) operated during the Archaean and, greenstone belts are allochthonous remnants of accreted oceanic crust, plateaus and/or island arcs¹⁻³. Most of the supporting evidences of this aspect are mainly based on the geothermal inferences, structural patterns and geochemical data from Canadian and South African greenstone belts. However, for the greenstone belts of Karnataka, India, the continental rift model was advocated^{4,5}, although oceanic crust-based origin of these belts has been demonstrated⁶⁻⁹. Recently¹⁰ it is suggested that the Dharwar greenstones were deposited in marginal or back arc volcano-sedimentary basins. These authors¹⁰ believe that the rocks in eastern Karnataka, including Sandur belt, formed as intra-arc basins above the evolving Dharwar batholith. Here we report evidence of horizontal compression, shearing and thrusting of volcanic rocks of oceanic origin and infer the consequent crustal shortening for the Sandur greenstone belt of Dharwar craton (Figure 1).

Sandur schist belt situated in the eastern margin of the Karnataka nucleus mainly consists of different types of metavolcanics and metasediments. The volcanic rocks are essentially tholeiitic basalts along with intermediate and acid volcanic rocks. These volcanics show well-developed pillow structures which on extensive deformation are elongated and stretched. The study of their stretching will provide a clue to the mechanism (process) of their deformation.

The pillow structures of the Deogiri and Donimalai formations at the western margin of the belt, south of Hospet, and south-west of Sandur (150 m thick across the strike) are stretched (Figure 2), whereas pillows of both the formations from the eastern part of the belt have normal length/width ratio (X'/Y') ranging from 1 to 2. More than 1200 pillows were measured at six places in the belt for their length, width, convexity and dip/inclination of the flow layers (Figure 1). Z' is exposed rarely and wherever exposed it is always less than X' and more than Y' ($X' > Y' > Z'$). The data are presented in the histograms depicting the variation in the L/W at each locality (Figure 3). All along the western margin of the belt, the pillows are compressed in NE-SW and stretched in N 40°-45° W direction. About 80% of the pillows have been stretched to give the length/width ratio ranging between 3 and 30, and 81% of the pillows have length/width ratio between 3 and 12 (Figure 3 a). The width (Y') of these pillows varies from 0.5" to 20", with 88% of the pillows having a width (Y') between 1" and 10" only. On the other hand, the length (X') of these stretched pillows varies from less than 10" to 190" (Figure 3 a). However, 72% of the pillows have length (X') more than 30" i.e. between 30", and 190". Most of them are stretched to such an extreme extent that they have a 'pencil shape' (Figure 2 a) appearance in a vertical or semivertical exposure where Z' is available. Some of the pillows have width between 4" and 6" and length between 40" and 60" with pointed ends. Large-scale variation in the overall size of the pillows even in stretched form is illustrated in Figure 2 b. Due to compression and metamorphism the recrystallization of original mineralogy into amphiboles and sodic plagioclase with development of well-defined axial plane schistosity has taken place (Figure 2 a). This schistosity is observed in individual pillows too. Although the texture and mineralogy show recrystallization, the deformed boundaries (rims) of the pillows are preserved with the general destruction of the convexity (Figure 2) and the flow layers have become almost vertical. A fracture system (cleavage) parallel to the compression direction has developed in most of the pillows. This indicates that the post-consolidation directional stress has been very effective on these pillows and other associated rocks (Figure 2 a) and continued to be active for a fairly long time. All along the belt the first generation axial plane cleavage (D_1 deformation) dips towards

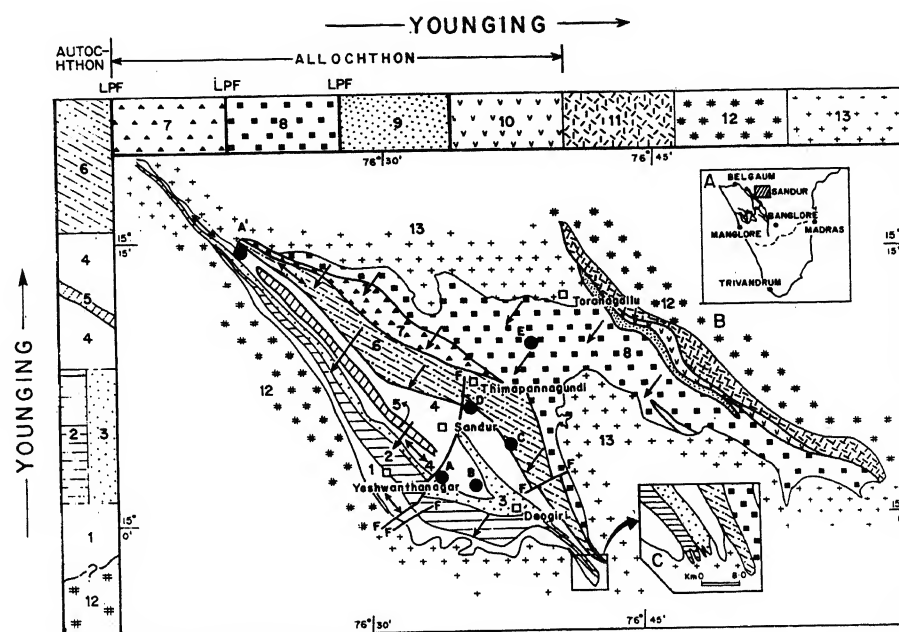


Figure 1. Geological map of the Sandur Greenstone (schist) belt, with a location map of India and the proposed stratigraphic succession. 1, Yeswanthanagar block (YB); 2, Deogiri block (DB interbedded with banded manganese formation); 3, Western volcanic block (WVB interbedded with banded iron formation); 4, Central volcanic block (CVB); 5, Greywacke; 6, Eastern volcanic block (EVB interbedded with banded iron formation); 7, North central acid volcanic block (NCAVB interbedded with active plate margin turbidites); 8, Sultanpura volcanic block (SVB); 9, Sediments of eastern volcanic block; 10, Eastern acid volcanic block (EAVB); 11, Amphibolites of eastern block; 12, Granitic gneisses and granites; 13, Younger granites.



Figure 2a, b. *a*, Stretched pillows, 5 km south of Hospet almost vertical plane. End of the individual pillows has become pencil shaped. F_1 axial plane schistosity and fracture cleavage are both visible; *b*, Stretched pillows from west of Sandur from spot A".

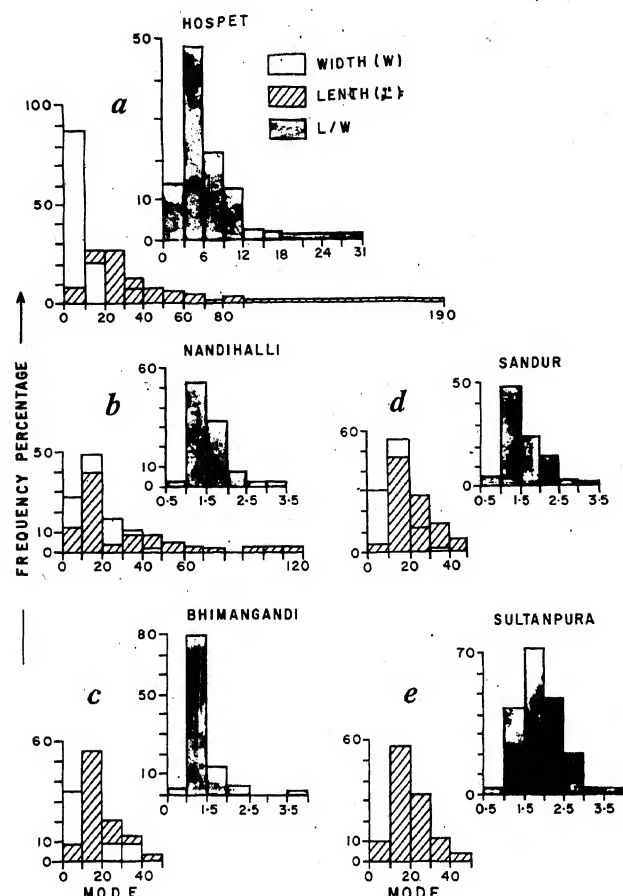


Figure 3a-e. Histograms showing the length, breadth and the length/breadth ratio of the pillows in different places in the Sandur schist belt.

north-east, indicating the compression direction (Figure 1). The pillows exposed in most of the eastern part of the belt do not exhibit this compression and stretching. Their length/width ratio generally remains between 1 and 2. Determination of length/width in the centre of the belt Nandihalli, south of Sandur (Figure 1, spot B) shows that 85.41% of the pillows have length/width ratio between 1 and 2, with 40% having length between 10" and 20" and 48% between 10" and 120" (Figure 3 b). There are giant pillows in this region which have length and width measuring up to 120" and 80", respectively. The relict mineralogy and igneous texture are found in many sections. In most of the sections, relict augite is found rimmed by amphibole. Plagioclase boundaries are not clear as the reaction between plagioclase and other minerals has not been completed. Chlorite, amphibole and epidote are found along these boundaries with fibrous development of actinolite being quite common.

Along the eastern part near Bhimangandi (Figure 1, spot C), 80% of the pillows have their length varying between 10 and 40", and 84% pillows have their width varying between 5 and 20" (Figure 3 c). Consequently,

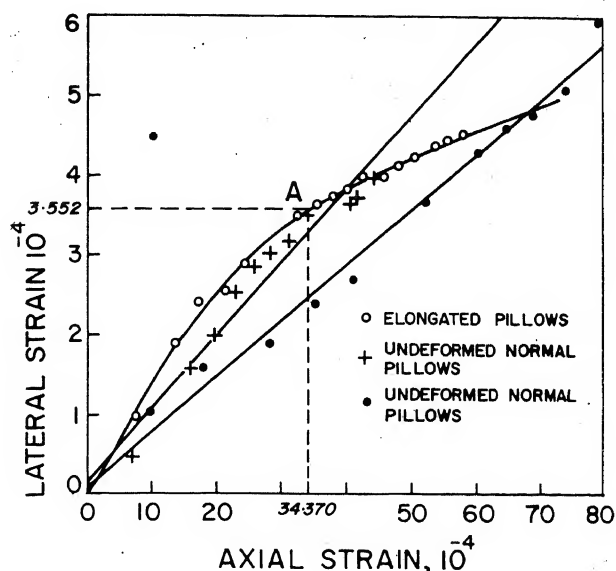


Figure 4. Poisson's ratio showing axial strain and lateral strain at 1.5 kb. It can be seen that up to 3.55 and 34.37 lateral and axial strain, the ratio maintains between 1:10 and 1:14, i.e. the range of the plastic deformation for these rocks. From point A onward, post-consolidation deformation is indicated, resulting in the elongation of the pillows.

92% of the pillows have length/width between 1 and 2. Similarly, west of Sandur (Figure 1, spot D) in the eastern volcanic block 83.61% of the pillows have a length varying between 10 and 40", and 93.44% of the pillows have width between 5 and 30" (Figure 3 d). Therefore, 90.53% of the pillows have length/width ratio between 1 and 3 and 57% of them have this ratio between 1 and 2. Seldom anywhere in the eastern part of the belt does the length/width ratio of the pillows cross 3.5 or 4. Further east, after crossing the Eastern volcanic block (EVB) in Sultanpura block (Figure 1, spot E) there is considerable variation in the length and width of the pillows (Figure 3 e). Here 48% of the pillows have length varying between 10 and 20" with a maximum length of 60". Width of these pillows ranges up to 40" with 56% of them ranging between 10 and 20". Most of these pillows show convexity towards east and 90% of them have length/width ratio between 1 and 3 (Figure 3 e). This shows that in the entire belt, the maximum deformation, elongation and compression have occurred along the western part of the belt. The average reduction in the width of the pillows is around 7 to 8 times and the increase in the length also appears to be of the same order. This shows that at least 7 to 8 times crustal shortening in the NE-SW direction has taken place along the western margin. Similar elongated pillows have been noticed in the Nondweni greenstone belt, southern Kaapval Craton, South Africa, where a shortening factor of 100 has been suggested¹¹. Such zones of elongation and stretching are zones of high strain parallel to strike direction and represent thrusting.

Elastic properties of the elongated and normal pillows under uniaxial stress of 1.5 kb have been studied to understand the mechanical behaviour of lateral strain to axial strain (Figure 4). In undeformed pillows, the slope between lateral and axial strain remained nearly uniform, while in the elongated pillows the slope is distinctly different. The strain measurements indicate that the elongated pillows are brittle in their behaviour and the strain ratio is approximately 1:24, otherwise this ratio in case of undeformed pillows is 1:10 or 1:14. This change in the strain ratio appears to have been caused by the post-consolidation deformation during shearing and thrusting (Figure 4). Brittle deformation and development of fracture system almost at 90° to the schistosity is observed in most of the pillows.

Pillows are exposed in almost all late Archaean greenstone belts of Karnataka and other parts of the world¹² and the length/width measurements at other places such as Gadag and Ingaldhal in Chitradurga schist belt have confirmed that the length/width ratio of the normal undeformed pillows generally remains between 1 and 2. This increase in the length/width ratio along with change in the dip of the flows from 0 to 90°, almost vertical F_1 fold axis and the 20°–80° NE dip of the first generation axial plane schistosity are unequivocal evidence of horizontal compression and large-scale movement of the rock mass. Recently the 35 km thickness of the Sandur belt has been attributed to the thrust thickening¹⁰.

Under the P–T conditions of regional metamorphism (confining pressure), elongation of pillows would not have taken place, as in the case of other parts of the belt (Spot B to E, Figure 1). This suggests that in addition to confining pressure the lavas of the western margin were subjected to a directional stress, which continued to produce post-consolidation brittle deformation. This may mean that the lavas of western margin were part of a subducting slab and compression continued till the closing of the proto-ocean. Therefore, it is argued that the western margin of the Sandur belt has been overridden by its eastern part. Furthermore, geometry and the wavelength (2–10 m) of the D_1 fold closures at the northern and southern termination of the belt¹³ substantiate the inference that horizontal compression and crustal shortening have been a very significant and frequent aspect of tectonic evolution of this greenstone belt.

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Palaeotectonic implication of Lamayuru lake (Ladakh)

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The age of ancient Lamayuru lake in Ladakh, Trans-Himalaya, has been speculative due to non-availability of proper date at the base of its sediment profile. A date of 35,000 ± 600 yr B. P. was reported by earlier workers in the upper part of lower sedimentary profile but precise timing of the development of lacustrine conditions is all important to know the origin of the lake and the question whether it was formed by normal geomorphic processes of slope derived debris damming or tectonically induced slope instability leading to river impoundment. The present study shows that the lake developed around 45,000 years ago, which coincides with a regional tectonic event in the Trans-Himalaya. The geomorphic processes were but adjunct to the intrinsic process of earth shaking during this episode of tectonic activity in the region. Timing of initiation and closure of lacustrine sedimentation in the Himalayan river valleys could offer an important clue for determining the recurrence interval of major earthquakes in the Himalaya. This calls for extensive work both in space and time.

THE late Quaternary remnant lacustrine deposits lying at an altitude of 3600 m in Lamayuru, Ladakh (Figure 1), comprise mudstone, siltstone and sandy shale facies

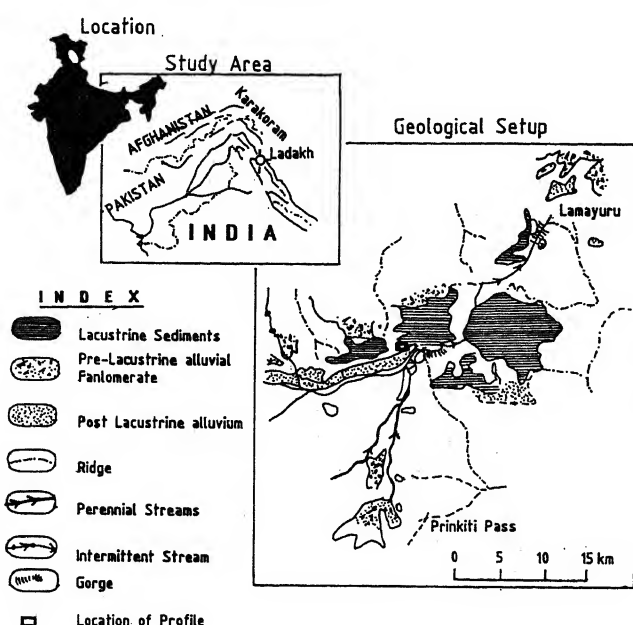


Figure 1. Geological map of the Lamayuru lacustrine sediments (Modified after Fort *et al.*³). The inset map shows location and the study area.

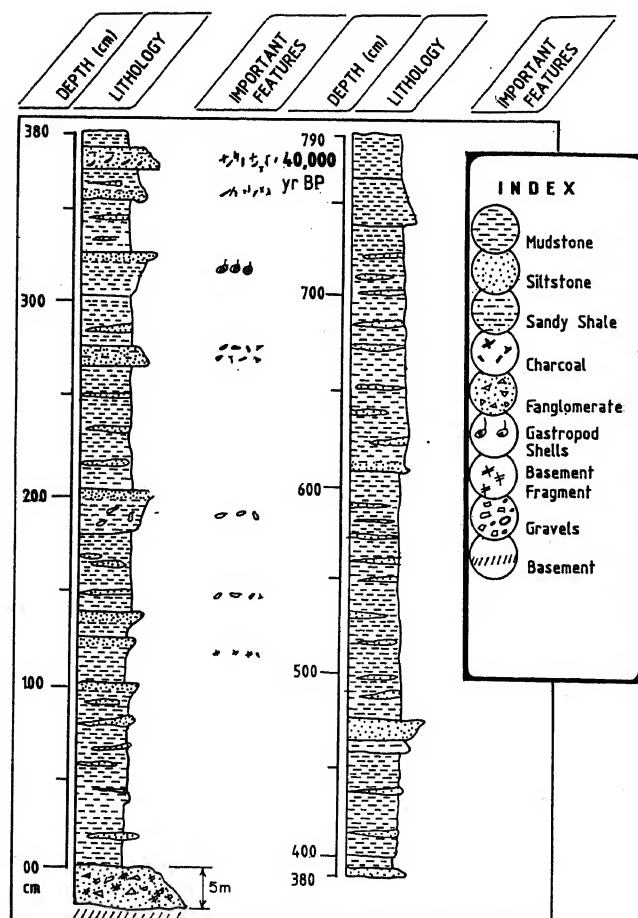


Figure 2. Lithosequence of Lamayuru lacustrine sediments at Rong-Gongkha.

resting over the prelacustrine fanglomerate. The sediments in the central part are rarely preserved due to extensive erosion and slumped blocks. The basement rocks are composed of argillaceous and calcareous Lamayuru flysch in the north, east and west of the basin, while the Tethyan carbonates are exposed in the south of the basin. The clay and silt contents of flysch have favoured post-lacustrine mass wasting processes including debris and rock slides in the proximal part of this ancient lake body. At present the climate of Lamayuru area is cold and dry and devoid of any vegetation except some scrubs.

In the present study, a 7.9 m profile of lacustrine sediments in the central part of the basin at Rong Gongkha has been investigated in detail (Figure 2). The base of the lacustrine fill lies on top of about 5 m thick fanglomerate which rests on the flyschoid basement rocks. The same profile was worked in detail by Sangode and Bagati¹ for palaeoclimatic studies using magnetic susceptibility and sedimentological parameters. The profile under investigation consists of five facies. These are: i) mudstone-siltstone, ii) mudstone-siltstone with charcoal, iii) mudstone-siltstone with small clasts, iv) sandy shale, and v) mudstone.

The mudstone-siltstone is the dominant facies occurring at various stratigraphic levels. In this facies mudstone is grey and shows parallel laminations. The thickness of laminae varies from 0.5 to 1 cm but increases in up section. The mudstone-siltstone with charcoal facies occurs at four stratigraphic levels. At places the charcoal is associated with mud pellets and is aligned parallel to the bedding. The mudstone is yellowish grey to ash-coloured and is generally laminated. The charcoal is generally associated with thick siltstone layers and shows grading. Some plant impressions were also observed in this facies. The charcoal fragments lying at 350–370 cm level were radiocarbon dated. The mudstone-siltstone with small clasts facies is present at two levels and shows grading. The clasts are generally associated with mudstone and oriented parallel to the bedding. Both intraclasts and extraclasts are present in this facies and represent turbidity deposits. The sandy shale facies is present at one level only in the upper part of the sediment profile. The laminae in this facies are 3–5 cm thick. It is yellowish grey and fine grained. The mudstone facies occupies the topmost part of the profile and shows parallel lamination.

In the central part of the basin the facies assemblage with charcoal and sedimentary structures indicate that density flows were responsible for deposition of silty turbidites. They are associated with sediments deposited under calm conditions and the deposition took place in a progressively deeper water body. The source for silty turbidites and sandy shale mudflows was probably from the Fotu La as indicated by the presence of large charcoal fragments within the lacustrine sediments exposed

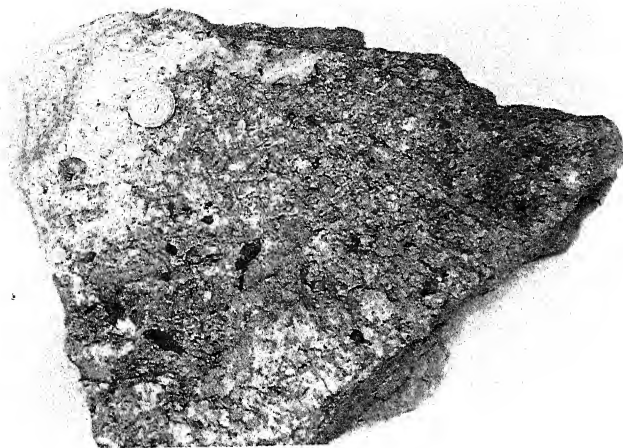


Figure 3. Siltstone-mudstone containing charcoal fragments (dark patches).

in upper reaches of the main Lamayuru channel. The presence of charcoal with plant impressions (Figure 3) indicates that the source was very close to the site of deposition. Bagati and Thakur² are of the opinion that during this period the climate was warm and forests grew luxuriously. Hence forest fires were common in the catchment. The warm phase is further supported by magnetic susceptibility study of the sediments¹.

The charcoal fragments recovered from siltstone-mudstone facies at 350–370 cm level were dated by the ^{14}C method (BS-879). The sample has yielded a date of 40,000 yr B. P., the maximum range provided by ^{14}C method. In essence, the sample could be much older. There is no other date available in the profile, hence the timing of lacustrine sedimentation could not be worked out directly. A date of $35,000 \pm 600$ yr B. P. given by Fort *et al.*³ does not correspond with the present profile at its equivalent height and level since their profile lies far away from our site of investigation. Thus, the only method that could be applied for determining the commencement of lacustrine condition is the rate of sedimentation, but again there are limitations posed by non-availability of another radiometric date in the profile. Since this is significant for many reasons, we have used average rate of sedimentation calculated for the Tsokar lacustrine sediments on the basis of radiocarbon dates for core TP-6 (Figure 4) (ref. 4). The basis being that Tsokar and Lamayuru fall in the same geographical entity or geomorphological domain, which permits logical application of known sedimentation rate of the former to the latter where such data do not exist. Such an approach is based on a work where empirical equation of sediment yield in relation to catchment characteristics has been used to estimate the distribution of sediment yield in adjoining catchments of similar morphology⁵.

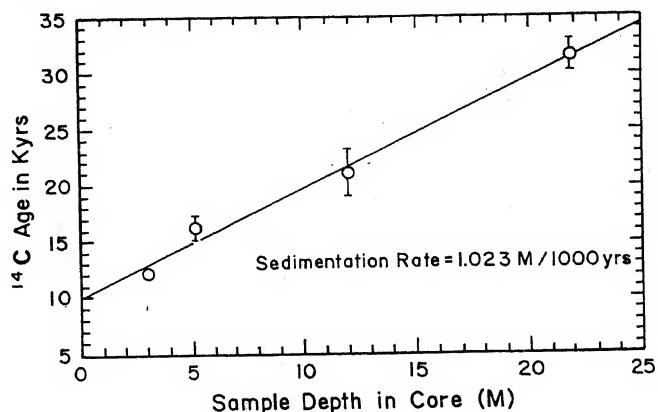


Figure 4. ^{14}C age vs depth of the sediment profile TP-6 from Tsokar lake. The rate of sedimentation, 1 m/1000 yr, is based on the linear regression line drawn through the data points (after Rajagopalan *et al.*⁴).

Thus by applying the sedimentation rate of Tsokar lake (approx. 1 m/1000 yr) to our profile from average point of 360 cm level where charcoal sample was recovered, we get a time frame of 3600 years to the base of the lacustrine sedimentation. Adding this figure to 40,000 yr B. P., we get a date of 43,600 yr B. P. for the commencement of the lacustrine phase. As Fort *et al.*³ postulate that the filling of the Lamayuru basin may have taken anywhere between 1000 and 10,000 years, we believe that attaining of full lacustrine conditions may have taken anywhere up to 2000 years or so. From this work we estimate that the initial damming of the erstwhile Lamayuru river took place probably somewhat prior to or near about 45,000 yr B. P. Due to the obliterated top sequence there is difficulty in calculating the closure of the lacustrine condition based on the rate of sedimentation. Hence more detailed work is required to answer this question. Assumptions based on discrete evidences, at this stage, have the risk of committing gross error.

The origin of the lake is attributed to tectonically induced slope instability in the basin, a sudden pulse of which mobilized large quantities of slope debris to dam the Lamayuru river. The episodic active nature of the lineament running parallel to the Lamayuru river is amply revealed in the narrow and deep cross profile developed after the depletion of the lake. The principal cause of blockade by periglacially derived debris as held by Fort *et al.*³ in this light appears substantially unconvincing. There are evidences where lake basins like Sumdo in lower Spiti valley were developed by high seismic activity in the region resulting in blocking of the river around 45,000 yr B. P. (ref. 6). Reactivation of the Nainital Fault around the same time⁷, though in a different tectonic domain, could be taken as an indirect clue for a major tectonic episode in the Himalaya. By analogy of

timing it is possible that the same event was responsible for the development of discrete lake basins in the Spiti valley⁸.

In conclusion it may be said that the Himalaya which registered its maximum uplift during Pleistocene experienced periodic regional tectonic impulses to account for impoundment of rivers, thereby leaving imprint of such evidences for posterity. The depletion of such lake basins could be either due to normal processes of erosion or triggering of post-blockade major tectonic impulses. Given this thesis, the timing of the depletion of lake basins on a larger regional scale assumes importance in determining the recurrence interval of catastrophic earthquakes in the Himalaya. Such an objective calls for extensive field work and dating of river valley lacustrine deposits along the length and breadth of the Himalaya.

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Chemical age of detrital zircons from the basal quartz-pebble conglomerate of Dhanjori Group, Singhbhum craton, Eastern India

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Chemical ages of detrital zircon concentrates from the uranium-bearing basal quartz-pebble conglomerate of Dhanjori Group from six localities have been determined based on high precision analysis of U, Th and Pb. Three of the zircons gave concordant ages of 3044–3090 Ma, which represent the minimum age of the provenance rock and older age limit of Dhanjori sedimentation. The Singhbhum Granite Complex dating between 3.3 Ga (Phases I & II) and 3.12 Ga (Phase III) are the likely provenance rocks. Chemical ages, coupled with evidences from field relations and temporal nature of uranium-bearing QPCs suggest that the sedimentary members of Dhanjori Group are likely to be older than the presently assigned age of 2300 Ma.

THE Singhbhum–Orissa craton, eastern India, comprises of a complex geological assemblage of Archaean and Proterozoic age. Chronostratigraphic status of many of

the lithogroups in this craton, especially the Dhanjori Group and Iron Ore Group (IOG) are still debatable^{1,2}, mainly because of inadequate radiogenic ages. The occurrences of uranium-bearing basal quartz-pebble conglomerates (QPC) in both Dhanjori Group³ and IOG⁴ are important in this context. Detrital U-bearing QPCs the world over have distinct temporal and stratigraphic positions^{5,6} and are generally confined to Archaean–Proterozoic transition period. Among the detrital heavy minerals in such conglomerates, zircons, because of their highly refractory nature, are considered provenance diagnostic. The ages of detrital zircon in QPCs correspond to the age of the parent rock and thereby aid in establishing provenance and stratigraphic position of the QPCs. In this communication, we report U, Th and Pb contents of detrital zircons from some QPC horizons at the base of Dhanjori Group and their chemical ages.

The volcano-sedimentary sequence of Dhanjori Group began with a basal quartz-pebble conglomerate, followed by a thick sequence of fuchsite-quartzite and an occasional phyllite. Upper part of the sequence is an extensive basic volcanic sequence. Basal QPC is exposed intermittently along the south, east and western margins of the Dhanjori basin. Conglomerate samples for the present study have been collected from six localities; Butgora in the western margin; Phuljari, Jawardih and Asthakaoli in the southern margin; and Tirioburu and Chakri in the eastern margin of the basin (Figure 1).

Zircon, chromite, rutile, monazite, pyrite, etc. are the prominent detrital heavy minerals found in all the QPC samples. Zircons recovered from these samples are characterized by their coarse (mostly 200–250 µm), stubby, unzoned nature and pink colour (Figure 2). Systematic morphometric analyses of zircon grains

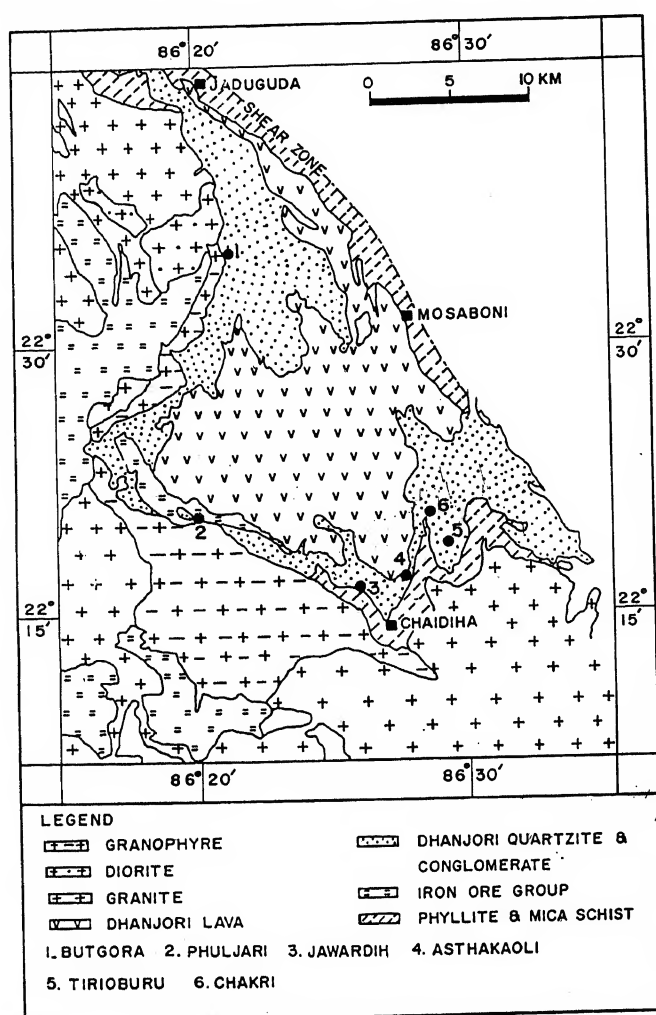


Figure 1. Geological map of Dhanjori basin (modified from Vasudeva Rao *et al.* 1988) showing the QPC sample locations.

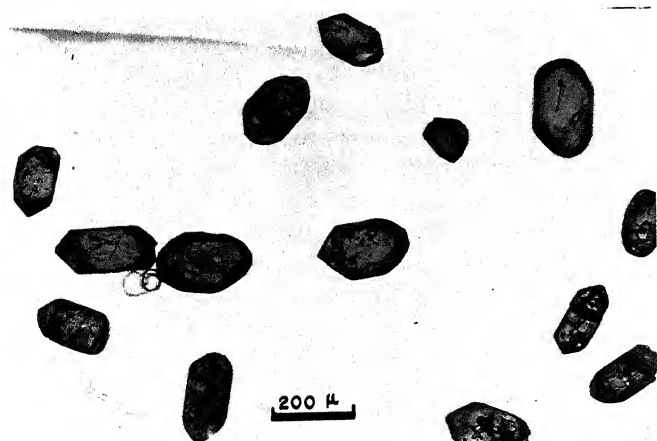


Figure 2. Detrital pink zircons separated from the QPC sample from Jawardih.

reveal their uniformity in colour, habit, length-breadth ratio (1.9 to 2.1), etc. Uniform morphometric characters of zircons from various locations suggest a common provenance for the Dhanjori sediments.

Zircon-rich heavy mineral concentrates were obtained from crushed and ground QPC samples by tabling, followed by sequential heavy media separation. Magnetic separation of these concentrates on Frantz Barrier Pole Isodynamic separator yielded a zircon-rich non-magnetic fraction devoid of monazite. The non-magnetic fractions from Butgora and Phuljari contained abundant pyrite. These pyrites were converted to oxides by heating the samples to 800°C in a muffle furnace. These oxides were removed by further magnetic separation. Stereomicroscopic handpicking was resorted to ensure near 99% purity of the zircon concentrates thus obtained. Special care was taken during stereomicroscopy so that no monazite remained in the zircon concentrate.

The pure zircon separates were finely powdered and treated with conc. HNO_3 followed by conc. H_2SO_4 to remove coatings and inclusions if any. U was analysed by delayed neutron activation analysis (DNAA) after irradiating the samples and standards in the CIRUS reactor (neutron flux $10^{13} \text{ n} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$) and by γ -ray spectrometry of the daughter product Np^{239} . Th was analysed by INAA after irradiating the samples in Apsara Reactor (neutron flux of $10^{12} \text{ n} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}$) for 4 h. γ spectra were taken using 125 cc HPGe detector coupled to PC based MCA system, having 30% efficiency with respect to $3'' \times 3''$ NaI detector and system resolution of 1.9 eV for 1332 keV of ^{60}Co . Pb was analysed by differential pulse anodic stripping voltametry. The errors in analysis were of the order of $\pm 5\%$ for U, $\pm 2\%$ for Th and $\pm 4\%$ for Pb. The U, Th and Pb content of the six samples of zircon are given in Table 1.

For calculation of chemical age, lead content in the sample is assumed to be of radiogenic origin by the decay of uranium (both ^{235}U and ^{238}U) and thorium. Non-radiogenic common lead content in zircons is shown to be as low as 0.01 ppm⁷; higher values of up to

Table 1. U, Th and Pb analyses of zircons from QPCs

Location	U (ppm)	Th (ppm)	Pb (ppm)	Th/U	Age (Ma)
Southern margin					
Jawardih	197	91	142	0.46	3090 \pm 124
Phuljari	148	70	80	0.47	2538 \pm 102
Asthakaoli	219	91	153	0.42	3048 \pm 122
Western margin					
Butgora	200	90	84	0.45	2109 \pm 84
Eastern margin					
Tirioburu	248	134	152	0.54	2745 \pm 110
Chakri	187	89	132	0.48	3044 \pm 122

Errors in estimations are $\pm 5\%$ for U, $\pm 2\%$ for Th and $\pm 4\%$ for Pb. Errors in age computation are $\pm 4\%$.

6.5 ppm have been reported only from rare magnetic zircons.

From the radioactive decay series of ^{238}U , ^{235}U and ^{232}Th , the atomic proportion of radiogenic lead in a mineral can be calculated⁸ using the following equation:

$$\text{Pb} = \text{U} \times [0.99276 (e^{\lambda_1 t} - 1) + 0.007196 (e^{\lambda_2 t} - 1)] + \text{Th}(e^{\lambda_3 t} - 1), \quad (1)$$

where U and Th are in atomic proportions. Modern abundance of ^{238}U is 99.276% of total U and that of ^{235}U is 0.7196%. The decay constants given by Jaffey *et al.*⁹ for ^{238}U ($\lambda_1 = 1.55125 \times 10^{-10} \text{ yr}^{-1}$, ^{235}U ($\lambda_2 = 9.8485 \times 10^{-10} \text{ yr}^{-1}$) and Th ($\lambda_3 = 4.9475 \times 10^{-11} \text{ yr}^{-1}$) are used in this calculation.

In this transcendental equation, t is the time duration in years and can be solved numerically. Bowles⁸ adopted an iterative approach of solution of this equation to calculate the age of uraninite. We have used the Newton-Raphson method of numerical analysis for solving this equation, for which the initial approximate age was obtained using the empirical equation

$$t = \ln \left(\frac{1.104 \text{ Pb}}{\text{U}} + 1 \right) \times \lambda_1^{-1}. \quad (2)$$

A computer program in FORTRAN has been written for solving eqn (1) by Newton-Raphson method (Appendix 1).

Chemical ages of the detrital zircons thus calculated are given in Table 1. Error in chemical age calculation is the cumulative manifestation of errors in chemical analysis, particularly of lead. Based on the errors in estimation of U, Th and Pb, the age calculated will have an error of $\pm 4\%$. Zircons from Jawardih, Asthakaoli and Chakri gave a very close age of 3090–3044 Ma. Tirioburu sample gave an age of 2745 Ma, whereas Phuljari and Butgora samples gave lower ages (2538 Ma and 2109 Ma respectively).

The lack of knowledge of non-radiogenic lead content leads to overestimation of age. But chemical ages calculated on individual uranium-bearing minerals having practically little initial lead content, using high precision analytical data^{8,10,11}, are found to be close to the isotopic age, and zircon is one such mineral. Since zircons from all the QPCs have similar optical and morphometric characteristics as well as similar U/Th ratios (see Table 1), it is reasonable to assume that their provenance is the same, and that the concordant age of 3044–3090 Ma obtained for three of the samples (Jawardih, Asthakaoli and Chakri) should be close to the age of the provenance rock. The lower ages of the samples from Butgora, Tirioburu and Phuljari are the result of natural lead loss or unintended lead loss that occurred during preparation of pure zircon separates. Though it is logical to assume that all the lead in zircon is radiogenic, post-crystalline lead loss, especially in broken zircon grains is definitely

a possibility¹², which would yield lower values in chemical age calculations. Accordingly, the concordant age of 3040–3090 Ma may be taken as the minimum age of the provenance rock. Isotopic age of zircons, and hence that of the provenance rocks, can be expected to be close to this age on the higher side, i.e., ≥ 3100 Ma, taking into account minor lead loss. The ≈ 3100 Ma, also represents the maximum age limit of commencement of sedimentation in the Dhanjori basin.

The heavy mineral suite of the Dhanjori QPCs is dominated by the presence of zircon, monazite and chromite. Quartz and quartzitic pebbles, abundance of detrital zircon and monazite, and geochemical characteristics of the QPCs including their REE pattern (paper under preparation) suggest the predominance of a granitic rock unit in the provenance for Dhanjori sediments. Detrital chromite suggests the occurrence of some ultramafic rocks also in the provenance. The palaeocurrent and basin analysis based on sedimentary structures, and textural maturity of the QPC, indicate a fluvial to deltaic depositional environment on a peneplained granite-greenstone terrain³. Among the probable older rocks of the Singhbhum craton that could be the source for zircons in the QPCs, the most widespread is Singhbhum Granite Complex belonging to three successive and closely related phases¹³. The other older rocks are the Older Metamorphic Tonalite Gneiss (OMTG) and Older Metamorphic Group (OMG). Singhbhum Granite phase III has been dated by Pb–Pb whole rock isochron method as 3130 ± 28 Ma and by Sm–Nd method as 3120 ± 100 Ma, while the Phase I is ≈ 3300 Ma old². OMTG rocks have been dated by Pb–Pb method as ≈ 3400 Ma¹⁴ or older¹⁵. The Pb–Pb age data on zircons from OMG¹⁶ gave ≈ 3550 Ma as the older age limit for OMG sedimentation. We have compared the morphometry of zircons in OMG samples from Champua and OMTG samples from Saraikela with those of Dhanjori QPCs and found that the Dhanjori zircons are distinctly different from those found in OMG and OMTG samples. On the other hand, several samples of Singhbhum Granite are found to contain appreciable amounts of zircon morphometrically similar to those found in Dhanjori QPCs¹⁷. It is reasonable to infer from these observations that the prominent source rock for Dhanjori Group sediments is Singhbhum Granite; however it is difficult to decipher at this stage whether Phases I and II (Singhbhum Granite A) are the major contributors or phase III (Singhbhum Granite B).

Dhanjori Group of rocks are assigned an age of ≈ 2300 Ma by Saha *et al.*¹⁸. However, the following considerations indicate that the sedimentary members of Dhanjori Group of rocks are likely to be older in age: (i) Uranium-bearing basal QPCs are generally confined to late Archaean-early Proterozoic transition period. (ii) The provenance rocks are of 3100 Ma or of older age. (iii) There is striking similarity in the geological

setting of QPC units of Dhanjori with Bababudans of Dharwar Super Group of Karnataka³, of age estimated¹⁹ to be between 3000 and 2800 Ma. The 555 m level crosscut of the Jaduguda uranium mine now exposes a continuous rock succession in the dip direction from Dhanjori metabasics through the uranium-bearing lodes to the schists and quartzites of Singhbhum Group. This conformable and continuous succession with Dhanjoris at the base also indicates that Dhanjoris are older than Singhbhum Group of rocks, an observation also made earlier by Mukhopadhyay²⁰ in the Rakha Mines. The Singhbhum Group of rocks has been assigned the age of c.2300 – 2400 Ma. It is also interesting to note here that the basal QPCs of IOG also contain detrital heavy minerals including zircons similar in character to those found in QPCs of Dhanjori. Because of these mineralogical similarities, we believe that the provenance of both IOG and Dhanjori Group was the same, and the sedimentation in IOG and Dhanjori basins was more or less coeval.

Appendix

C NEWTON-RAPHSON METHOD FOR DETERMINATION OF
CHEMICAL AGE BY

C SOLVING EQUATION FOR RADIOGENIC LEAD

```

C
    REAL LU238, LU235, LTH
    CHARACTER NCODE*10
C
    NCODE = SAMPLE CODE
    OPEN (UNIT = 15, FILE = 'CHEMAGE.OUT', STATUS =
        'OLD')
    WRITE (*, 5)
5
    FORMAT (10X, 'Please Enter the Sample Code:')
    READ (*, *) NCODE
    WRITE (*, 10)
10
    FORMAT (1X, 'Enter the increment in Time and Accuracy
        check value',/)
    READ (*, *) DELT, EPSI
    U238 = 0.99276
    U235 = 0.007196
C
    U238 AND U235 ARE CRUSTAL ABUNDANCE OF THE
        ISOTOPES
    WRITE (*, 20)
    READ (*, *) U1, TH1, PB1
C
    U1, TH1 & PB1 = CHEMICAL ASSAYS OF U, Th & Pb IN
        THE SAMPLE
20
    FORMAT (1X, 'Enter the assays of U, Th, Pb')
C
    U = U1/(U238*238 + U235*235)
    TH = TH1/232
    PB = PB1/206
C
    U, Th & Pb = ATOMIC PROPORTIONS OF U, Th, AND Pb
        IN THE SAMPLE
    LU238 = 1.5513E-10
    LU235 = 9.8485E-10
    LTH = 4.9475E-11
C
    LU238, LU235 & LTH = DECAY CONSTANTS OF U238,
        U235 AND Th
    TA = (LOG(1.104*PB/U + 1))/LU238
    T = TA
C
    FOFT=U*(U238*(EXP(LU238*T) - 1) + U235*
        (EXP(LU235*T) - 1)) + TH*(EXP(LTH*T) - 1) - PB

```

```

C
30
    TN = T-DELT
    FOFTN = U*(U238*(EXP(LU238*TN) - 1) + U235*
        (EXP(LU235*TN) - 1)) + Th*(EXP(LTH*TN) - 1)
    PBC = FOFTN
    FOFTN = FOFTN-PB
    WRITE (*, 35) FOFTN
35
    FORMAT (10X, 'Pb(Calc) - Pb(Exptl) = ', E15.4/)
    IF (FOFT*FOFTN) 70, 40, 60
40
    TNF = TN
    WRITE (*, 50) TNF, FOFTN
50
    FORMAT (10X, 'AGE IS = ', E15.6, 10X, 'ERROR = ',
        E15.6/)
    STOP
60
    T = TN
    GOTO 30
70
    FOFTN = U*(U238*(EXP(LU238*TN) - 1) + U235*
        (EXP(LU235*TN) - 1)) + Th*(EXP(LTH*TN) - 1)
    PBC = FOFTN
    FOFTN = FOFTN-PB
    DFOFTN = U*U238*LU238*EXP(LU238*TN) + U*U235*L
        U235*EXP(LU235*TN) + Th*LTH*EXP (LTH*TN)
    TN1 = TN-FOFTN/DFOFTN
    IF (ABS(TN1-TN) - EPSI) 90, 90, 80
80
    TN = TN1
    GOTO 70
90
    TNF = TN1
    FOFTN1 = U*(U238*(EXP(LU238*TN1) - 1) + U235*
        (EXP(LU235*TN1) - 1) + Th*(EXP(LTH*TN1) - 1)
    PBC = FOFTN1
    FOFTN1 = FOFTN1-PB
    WRITE (*, 25) TA
25
    FORMAT (10X, 'Approximate age in years = ', E15.4/)
    WRITE (*, 50) TN1, FOFTN1
    ERROR = PBC - PB1
    WRITE (15, 200) NCODE
200
    FORMAT (25X, 'The Rock Sample code:', A10//)
    WRITE (15, 250)
250
    FORMAT (10X, 'Element', 12x, 'U', 14x, 'Th', 14x, 'Pb')/
    WRITE (15, 300) U1, Th1, Pb1
300
    FORMAT (10X, 'Assay in ppm', 3(F10.3, 5X)/)
    WRITE (15, 25) TA
    WRITE (15, 400) PBC
400
    FORMAT (10X, 'The Pb calculated (atomic prop.) = ',
        E15.7)
    WRITE (15, 500) PB
500
    FORMAT (10X, 'Experimental value of Pb (atomic.
        prop) = ', E15.7)
    WRITE (15, 600) ERROR
600
    FORMAT (10X, 'The Error = ', E15.7)
    WRITE (15, 700) TNF
700
    FORMAT (10X, 'The calculated chemical age in years = ',
        E15.7//)
    STOP
    END

```

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Over the years, the editors and authors of the *Annual Review of Physical Chemistry* series have attempted to provide us with a broad definition of physical chemistry in an era of growing international collaborations and subsequent blurring of the sharp boundaries between disciplines. The present volume starts with reminiscences of a spectroscopist, William Klemperer who describes how physical chemistry (read spectroscopy) has grown in front of his and his colleagues' eyes at Harvard. He narrates how rotational spectroscopy in cold molecular beams has helped us understand interstellar chemistry. This introductory chapter, although well written, is more of a technical account than personal. We wonder if a scientist only remembers the scientific achievements of the past and not the personal incidents that were associated with it along with the personalities!

Most of the articles in the present volume deal with gas phase spectroscopy and dynamics of one kind or other. Herbst as well as Sims and Smith have dealt mainly with rates of gas phase reactions at low temperatures. Herbst's motivation being guided by chemistry in interstellar medium, he concentrates on ion-molecule reactions for generating exotic species and rules out the possibility of neutral-neutral reactions at the low temperature and density of the interstellar medium. Sims and Smith, on the contrary, discuss elementary chemical reactions of neutral reactants at temperatures as low as 13 K. Several examples of molecular energy transfer at low temperatures are discussed.

Gas phase photochemistry and dissociation/reaction dynamics are described, at least, in four experimental and five theoretical chapters. In his article, Bernstein describes intracluster electron and proton transfer, vibrational relaxation, predissociation and radical additions with suitable examples. Farrar has discussed ionic reactions with a special emphasis on proton and hydrogen atom transfer reactions of the atomic oxygen

anion. Gas phase S_N2 reactions have also been included. Mode-selective enhancement of chemical reactions of acetylene and ammonia cations with other species have been reviewed. Recent developments on two-dimensional imaging of a chemical reaction as it proceeds has been reviewed by Heck and Chandler. Quantum state distribution of a product and the velocity correlated to that distribution can simultaneously be measured by this new method. The authors present in detail how to convert the image into useful information. The instrumentation aspect has been emphasized so that the readers can follow the procedure with ease. This is an excellent article and discusses both orientation and alignment in uni- and bimolecular reaction processes. In another article, Loesch has reviewed recent progress in reactive beam collisions with special importance to orientation and alignment. Two techniques have been described, a brute force technique and an infrared laser-based optical technique, to orient and align molecules in a supersonic beam. The reactions of alkali metals with HF, ICl and CH_3X ($X=I$ or, Br) are discussed along with the relevant theoretical results.

Theoretical studies of polyatomic reaction dynamics have been illustrated by Bowman and Schatz. In this account, the authors have concentrated on four or more atom reactions. Currently, all efforts on experimental and theoretical fronts are focused on four-atom reactions in order to unravel the mechanism of these reactions. This is a timely review dealing with quantum and quasiclassical methods of calculating reaction rates and cross-sections. Adequate comparisons between theoretical and experimental results have been made in order to put the concerted world-wide efforts in this field in perspective. An equivalent but different approach of modelling three and four atom chemical reactions has been described by Jackson. The wave packet reactive scattering approach pioneered by Heller and improved by many has been discussed. Extension of the wave packet dynamics calculations to gas-surface reactions has been dealt with. In another article, Jolicard has discussed yet another method, the effective Hamiltonian for molecular dynamics calculations. This method, developed in the 1960s gained a lot of attention in the '70s. The

author has discussed the theoretical foundation in detail. Application of vibrational energy redistribution and photodissociation of van der Waals molecules, as well as femtosecond dynamics have been illustrated with examples. This is no longer a popular method and thus, the author specifically mentions the advantages offered by this method in his concluding remarks. The accuracy of full-quantum calculations in predicting spectra of hydrogen molecular cation and its isotopic variants has been reviewed by Leach and Moss. The hydrogen molecular ion is simple in the sense that electron correlation is not of concern in this species and a full quantum calculation can be carried out. The authors have deliberately limited the scope of this article to hydrogen molecular ions and stressed on the accuracy required to reproduce experimental numbers. This article will perhaps help the specialists in the field to learn the details of such calculations. Another theoretical article in this volume deals with analysis of molecular spectra. The excellent review by Kellman about algebraic methods in analysing spectra will benefit a large number of experimentalists. He describes the standard spectroscopic Hamiltonian as well as the more recent Lie algebraic techniques for fitting a detailed excited state spectrum. The application of algebraic Hamiltonians to complex spectroscopic problems has great advantages for the numerical task of fitting a spectrum, in comparison with trying to fit the spectrum to a potential energy surface. We hope that experimental spectroscopists critically evaluate this advantage!

Femtosecond pulse shaping and optical control have been discussed by Kawashima, Wefers and Nelson. This is a highly technical article, but we suppose, it would be helpful to those who are interested in coherent excitation in a short time-scale! Impulsive stimulated Raman scattering studies of coherent lattice vibrations in the solid state and impulsive one-photon absorption in atomic vapour have been discussed. Efficient photoinduced electron transfer in supramolecular and photosynthetic systems has been described by Franzen and Martin. They have included time-resolution of excited state vibrational motion, the electric field dependence of the quantum yield, and resonance Raman

and hole-burning experiments that probe the nature of the initially prepared excited state.

Laser cooling of neutral atoms to form a bound dimer between two free atoms has been discussed by Lett, Julienne and Philips. This branch of high resolution molecular spectroscopy has made significant progress in the past few years and this article will be a good starting point for a newcomer in the field.

With the recent development of zero-kinetic energy (ZEKE) photoelectron spectroscopy as well as resonance enhanced multiphoton ionization (REMPI) sub-wavelength resolution in photoelectron spectroscopy are routinely achievable and there have been numerous reports of rotationally resolved photoelectron spectra of light molecules. Wang and McKoy review the theoretical framework to understand the experimental results. Central to the understanding of the rich dynamics of rotational quantum state specific studies of molecular photoionization are a number of dynamical issues such as strong *l*-mixing in the molecular photoelectron continua, parity selection rules, partial wave mixing and interference. The article also reviews the experimental results of the rotationally resolved PES of a number of small molecules.

The infrared reflections absorption spectroscopy (IRRAS) technique is now no longer restricted to the study of well defined surfaces and more complex systems are being studied by this technique. IRRAS has been applied to investigate monolayers formation at the air-water interface. Mendelsohn, Brauner and Gericke review both the experimental and theoretical status of the field. Based on their survey the authors predict that the time is ripe for additional application of IRRAS especially to biological problems – interfacial enzyme catalysis, pulmonary surfactant functions, etc.

While experimental surface chemistry is moving towards an era where more complex systems are being studied, theoretical models still have a long way to go. While there are numerous reports on theoretical and computational investigations of adsorption of small molecules on well-defined surfaces, concomitant studies on the reactions of these adsorbed molecules have been few and far apart. This is partly because microscopic simulations, classical molecu-

lar dynamics or quantum scattering, require accurate potential energy surfaces. Garrison and Srivastava have reviewed the various recipes for generating many body reactive potential energy surfaces as well as their application. The potential energy surfaces described are for specific systems, the group IV elements and metals. The authors conclude by emphasizing the need for the development of new functional forms for multicomponent reactive systems.

Nuclear magnetic resonance continues to be an exciting technique for structure elucidation especially of biomolecules. In the last few years, an almost bewildering array of sophisticated new pulse experiments have been developed. Pelton and Wemmer describe the development of heteronuclear pulse sequencing experiments wherein the chemical shift of a proton is correlated with heteronuclear (^{15}N , ^{13}C or ^{31}P) chemical shifts. The technique has allowed investigation of protein and nucleic acid structures in the 10–40 kDa range in contrast to homonuclear ^1H methods which are limited to systems less than 10 kDa in size. Coulombic interactions and salt effects in nucleic acid solutions have been described in an article by Anderson and Thomas Record, Jr.

Nanochemistry is an emerging discipline within physical chemistry. Primary interest has focussed on the novel electronic structure and reactivity properties of small particles. Recent advances in the preparation and characterization of nanocrystals have also helped in addressing one of the long-standing questions of how large a system should be before it can exhibit a true thermodynamic phase transition. Any explanation or investigation would have to eliminate or establish the role of surface energy of these particles. The article on high pressure structural transformations in CdSe, CdS and Si nanocrystals by Tolbert and Alivisatos is quite timely. They review the experimental situation on solid-solid structural transformations in particles with different sizes and conclude that the observed elevation in the structural transition pressure is kinetic rather than a thermodynamic phenomenon.

In an excellent article, Yang and Parr describe various new developments in the density functional theory for calculating electronic structure of molecules. The goal of the article is set out clearly in the introduction. Recent contributions

to the basic theory, extension of the applicability to very large molecules and the understanding of chemical reactivity have been considered for discussion. This article is timely since this field is seeing a tremendous recent surge in popularity among chemists. The accuracy achievable currently using this method has been compared with that of conventional quantum chemical calculations for computing ground states.

Nucleation plays a key role in many natural phenomena, from crystallization in melts to particle formation in stratospheric clouds. It is not surprising that the study of homogenous and heterogenous nucleation is an old subject. Recent developments in both theory and experiment have given rise to a resurgence of interest in nucleation. Experimentalists, today, can measure actual nucleation rates from 10^{-4} to $10^5 \text{ cm}^{-3} \text{ s}^{-1}$ as well as probe the molecular content of these nucleating clusters using a variety of techniques. Experiments have gone hand-in-hand with progress in large-scale molecular dynamics simulation of the nucleation process. These developments have now made it possible to simulate both by experiment and computation, many of the particle formation phenomena occurring in the atmosphere. The article by Laaksonen, Talnaker and Oxtoby reviews all aspects of nucleation, experiment, theory and applications and provides a reasonable introduction to the subject for the non-initiate.

Finally, we have one criticism about the organization of this volume. No particular format has been followed by the authors while writing the reviews. As a result while some articles give a broad perspective of a field, others are narrow and limited in scope. All articles, however, carry important references in the field for the readers. This volume of *Annual Review of Physical Chemistry* certainly covers current topics and will be a useful addition to all libraries and groups working in the area of physical chemistry/chemical physics.

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Oxidative Stress and Aging. R. G. Cutler, L. Packer, J. Bertram and A. Mori, eds. Birkhauser Verlag, P. B. No. 133, CH-4010, Basel, Switzerland. 1995. 396 pp. Price: US\$ 124.

I am never keen on reading a book on proceedings of a symposium or contributing an article for one such. I feel that the material therein is old, repetitive and sketchy. In most cases they reflect the author's viewpoint and in some cases a way of bypassing the stranglehold of difficult peers. The authors would be delighted to give the invited talks and participate in the discussions, but to provide a written text is another matter. It is a task for the editors to collect the articles in time. The present book had proved these beliefs wrong. The first international conference on oxidative stress and aging was held in Hawaii, USA in 1994 and this book published in the next year reflects its deliberations. The editors say that they had to select some articles out of many more received. The subject was covered well, the articles are uniformly informative and current. The most useful item in the book is the list of references including the titles, mostly from the last 10 years indicative of the novelty and burst of activity of the field.

The 35 articles are grouped in related areas under the following titles:

oxidative stress and cellular senescence, genetic stability and damage, DNA repair genetics and life span, protein and lipid oxidation, mitochondria, age-related diseases and cancer, neuro-degeneration, nutrition, nitric oxide. Two introductory articles by two of the editors gave a preview of the two parts, oxidative stress and aging. These are recommended for study to get an idea of the status of this field of study. The articles covered too many subjects such as apoptosis, vascular smooth muscle cell proliferation, DNA damage and repair, poly ADP-ribose polymerase, muscle damage, diabetes, carcinogenesis, Parkinson's disease, dietary antioxidants and reactive oxygen species including nitric oxide. The articles provide frontier information on the current research activities focussing on the free radical theory of aging. The recent findings would have found a place in one or the other article. Thus I believe this book will be of value for the workers in the field and those who want to be initiated.

I have noticed band-wagon spirit had set in already. Beliefs are overtaking experiments and form the basis of theories, mostly correct albeit some unproven. Some examples are NAD-depletion cause of cell death, superoxide radical 'damaging in itself', 'most destructive process initiated by hydroxyl radicals is lipid peroxidation', 'longevity determi-

nant processes', importance of cellular GSH and vitamin E as antioxidants, cellular damage of macromolecules inevitable, SOD, catalase and glutathione oxidase are antioxidant enzymes, life-span proportional by their cellular concentration (given on cover illustration), contradictory reports on anti-cancer effect β -carotene, dietary restriction prolongs life, tea-polyphenols powerful antioxidants.

On such articles and books, I look for illustrations that unify information from many publications, catch the eye and leave a strong impression. In this I was disappointed. Some of these, eg. pages 31 and 102, do not convey the message and the one before the preface page lists all the 'actors' but not the 'action'. The block on 'hydrogen peroxide removal' on page 3 has unbalanced equations - H_2O could have been shown as a product for glutathione peroxidase and O_2 should not have been shown as a substrate for catalase. I also found that summaries are printed in a very small print almost unreadable. Notwithstanding these, I recommend this book to those workers interested in the field of oxidant stress.

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The cakravāla method

S. Raghavan

The object of this article spurred by a book review in *Current Science* (1996, 70, 753–754) is to try to place in proper perspective the well-known work of Indian mathematicians especially their kuttaka, bhāvanā and cakravāla methods evolved in connection with the solution in integers of certain indeterminate equations of degree one or two. In this effort, we base ourselves completely on André Weil's masterly, unbiased and incisive analysis of this topic in his beautiful book *Number Theory – An Approach Through History – From Hammurapi to Legendre*¹. Any help needed to understand related results concerning continued fractions can be readily secured from the book *An Introduction to the Theory of Numbers*² (especially chapter 7), although we endeavour to skirt a reference thereto by the reader by providing as self-contained an account as possible of facts related to simple continued fractions (in the sequel).

Solving indeterminate equations of the first degree, say $ax + by + cz + \dots = m$ in the variables x, y, z, \dots with integral coefficients a, b, c, \dots for integer values of the variables has been the key to cracking puzzles or finding integral solutions of simultaneous simple linear congruences, e.g. $x \equiv k \pmod{r}$, $y \equiv l \pmod{s}$ requiring $x - k, y - l$ to be divisible by integers r, s respectively for given integers k, l . A single linear congruence $px \equiv m \pmod{q}$ in the variable x for the modulus q is equivalent to a linear equation $px - qy = m$ in two variables x, y . Euclid's algorithm for finding the greatest common divisor of the integers p and q provides a method of solving the last-mentioned equation; expanding the rational number p/q (for $q \neq 0$) as a simple continued fraction gives an alternative (but equivalent) approach to the same problem. A clear description of the general solution of this linear equation in x, y can be found in the Sanskrit text *Aryabhatīya* of the 5th or 6th century A.D. In subsequent Sanskrit treatises, this method came to be known as the 'kuttaka' (= 'pulveriser') and is indeed a kind of forerunner of

Fermat's powerful principle of 'infinite descent'. It is also the 'first ever explicit description' of the general solution from anywhere, not taking into account China. Indian astronomy at that time was under the influence of Greek sources and yet perhaps one cannot with certainty attribute 'kuttaka' to Greek mathematics. In utter disregard or (possible) ignorance of this Indian dimension to the 'kuttaka' and of the connection with the seventh book of Euclid's *Elementa*, Bachet inserted a strong claim to the method as his own, in the second edition of his book on *Problèmes plaisants et délectables*.

Let N be a natural number which is not the square of an integer; the simplest example is $N = 2$. In view of the connection with finding close approximations to the irrational number \sqrt{N} by rational numbers, indeterminate equations of degree 2 like $x^2 - Ny^2 = \pm m$ for given integers m must have been indeed investigated by the Greek mathematicians. Special cases of the 'composition formulae'

$$(x^2 - Ny^2)(z^2 - Nt^2) = (xz \pm Nyt)^2 - N(xt \pm yz)^2, \quad (1)$$

e.g. for $N = 3, z = 5, t = 3$ may have been applied by Archimedes for finding rational approximations to $\sqrt{3}$. However, the composition formula (1) occurs explicitly in the work of Brahmagupta (in the 7th century) while seeking the solution in integers x, y of equations of the form $x^2 - Ny^2 = \pm m$ for any fixed natural number m . For $m = 1$, the name Pell's equation has come to stay for the diophantine equation

$$x^2 - Ny^2 = 1. \quad (2)$$

Such equations 'do occur in Diophantus...', but it is a rational solution that is asked for, even when accidentally a solution in integers is obtained...'. It may be reasonable to suppose that Archimedes was interested in equations of this type or, to be very optimistic, that he had even found a general method of solving equations like (2).

In the book entitled *Algebra with Arithmetic and Mensuration, from the Sanscrit of Brahmagupta and Bhāscara* by H. T. Colebrooke, one finds an entire section dealing with Brahmagupta's investigations (in the seventh century) on Vargaprakṛti – the solution of equations $Ny^2 + m = x^2$ in integers x, y with N as above and m , a non-zero integer; N is called 'gunaka' (or 'prakṛti') and m the ksepa (= additive). If the triple $(x, y; m)$ stands for a solution in integers x, y of the equation $x^2 - Ny^2 = m$, keeping N fixed all the while, the composition formula (1) is given by Brahmagupta in the form

$$(x, y; m) \cdot (z, t; n) \rightarrow (xz \pm Nyt, xt \pm yz; mn).$$

These laws acquired, in the post-Brahmagupta manuscripts in India, the name bhāvanā ('production') rules; in modern parlance, this is just the 'multiplicativity of the norm'. Brahmagupta shows, how composition of $(x, y; m)$ with a triple $(p, q; 1)$ gives a triple $(x', y'; m)$ for the same additive m . Under composition with itself, any triple $(x, y; m)$ yields a solution in rational numbers $x'/m, y'/m$ of the equation $(x'/m)^2 - N(y'/m)^2 = 1$ and indeed a triple $(x'/m, y'/m; 1)$ if x'/m and y'/m are actually integers. Applying then his bhāvanā, Brahmagupta solves equation (2) for several cases of N including ones like $N = 92$ or $N = 83$. For $m = -1$ or ± 2 , composition of a triple $(p, q; m)$ with itself leads to $(p^2 + Nq^2, 2pq; 1)$ or $((p^2 + Nq^2)/2, pq; 1)$ respectively.

Despite the remarkable results of Brahmagupta, the general solution of (2) is still not at hand. Actually, the cakravāla ('cyclic' method) for getting the general solution of equation (2) is to be found much later, around the twelfth century, in the work of Bhāskara; nearly the same description of the cakravāla is provided in a commentary of the eleventh century by 'an otherwise unknown author' Jayadeva, leaving one to guess who was the true inventor of the cakravāla. Following Weil, we can see how the brilliant cakravāla arises in a

natural manner from the work of earlier Indian mathematicians.

Starting from a triple (p_0, q_0, m_0) with 'small' m_0 , the idea is to use the bhāvanā to get a triple (p_1, q_1, m_1) with m_1 also 'small' and eventually hope to hit upon a triple $(u, v, 1)$ giving a non-trivial solution of equation (2), of course. First we can assume, without loss of generality that the greatest common divisor d of p_0 and q_0 in the initial triple is already equal to 1, since if $d > 1$, we could start instead from $(p_0/d, q_0/d, m_0/d^2)$. Since p_0 and q_0 have greatest common divisor 1, so have q_0 and m_0 clearly. Then the kuttaka readily enables us to find an integer x_0 such that m_0 divides $p_0 + q_0x_0$ (i.e. solve the arithmetical congruence $q_0x_0 \equiv -p_0 \pmod{m_0}$). If, in addition, the chosen x_0 is fixed in its residue class modulo m_0 so as to satisfy the inequalities $x_0 < \sqrt{N} < x_0 + m_0$, then we see that $\sqrt{N} + x_0$ cannot be negative provided that $|m_0| < 2\sqrt{N}$ (since ' $\sqrt{N} + x_0 < 0$ ' would imply that $2\sqrt{N} < \sqrt{N} + x_0 + |m_0| < |m_0|$) and consequently for $|m_0| < 2\sqrt{N}$,

$$0 < N - x_0^2 = (\sqrt{N} - x_0)(\sqrt{N} + x_0) < |m_0|(\sqrt{N} + \sqrt{N}) = 2|m_0|\sqrt{N}. \quad (3)$$

Composition of (p_0, q_0, m_0) with $(x_0, 1, x_0^2 - N)$ gives rise to the triple (p_1, q_1, m_1) where

$$\begin{aligned} p_1 &:= (p_0x_0 + Nq_0)/m_0, \\ q_1 &:= (p_0 + q_0x_0)/m_0, \\ m_1 &:= (x_0^2 - N)/m_0 \end{aligned} \quad (4)$$

are clearly integers. (In fact, by composition, $(m_0p_1)^2 - N(m_0q_1)^2 = m_0(x_0^2 - N) = m_0^2m_1$ and $q_0^2(x_0^2 - N) = q_0^2x_0^2 - p_0^2 + m_0 = m_0((q_0x_0 - p_0)(q_0x_0 + p_0)/m_0 + 1)$ is divisible by m_0 , i.e. $x_0^2 - N$ is divisible by m_0 in view of the greatest common divisor of m_0 and q_0^2 being 1 by our assumption above. Thus m_1 is an integer while the congruence condition on x_0 implies that q_1 is an integer as well and so are $p_1^2 = Nq_1^2 + m_1$ and p_1 too as a consequence!) Moreover, $|m_0m_1| = N - x_0^2 < 2|m_0|\sqrt{N}$, by (3) and therefore we have

$$|m_1| < 2\sqrt{N}. \quad (5)$$

Starting with the triple (p_0, q_0, m_0) , the passage to (p_1, q_1, m_1) as above gives an inductive construction of the triples (p_i, q_i, m_i) as follows.

It is convenient to take $q_0 = 1$ and $P_0 := [\sqrt{N}]$, the largest integer not exceeding the (positive) square root $[\sqrt{N}]$ of N , so that $0 < \sqrt{N} - p_0 < 1$. The congruence condition on x_0 now looks simpler, viz. $x_0 \equiv -p_0 \pmod{m_0}$ with $m_0 := p_0^2 - N < 0$, i.e. x_0 is any integer such that $x_0 + p_0$ is divisible by m_0 (but subject to the additional conditions $x_0 < \sqrt{N} < x_0 + |m_0|$, of course!). Due to the special choice of q_0 , the kuttaka does not need to be invoked here (for solving a congruence for x_0) but also at every subsequent step under the induction, as nicely emphasized by Weil. We reproduce his comments in this regard verbatim¹. 'Strangely enough, this does not seem to have been noticed by any of our Indian authors (nor even by their later commentators, down to the sixteenth century); they make no mention of it, and invariably refer to the kuttaka for the choice of x , even though their abundant numerical evidence could easily have convinced them that this was unnecessary.'

Let us assume the triples (p_j, q_j, m_j) , x_j for $0 \leq j \leq i$ constructed inductively with $|m_j| < 2\sqrt{N}$, $x_j \equiv -x_{j-1} \pmod{m_j}$ i.e. $x_j + x_{j-1}$ divisible by m_j , $x_j < \sqrt{N} < x_j + |m_j|$, $m_{-1} := 1$, $x_{-1} := p_0$ and $x_{j-1}^2 - N = m_{j-1}m_j$ for $j \geq 0$. Then composition of (p_i, q_i, m_i) with the triple $(x_i, 1, x_i^2 - N)$ leads to the definition of $p_{i+1}, q_{i+1}, m_{i+1}$, viz. $p_{i+1} := (p_ix_i + Nq_i)/m_i$, $q_{i+1} := (q_ix_i + p_i)/m_i$ and $m_{i+1} := (x_i^2 - N)/m_i$. The congruence condition on x_i above coupled with the relation $-q_ix_{i-1} + p_i = -x_{i-1}(q_{i-1}x_{i-1} + p_{i-1})/m_{i-1} + (p_{i-1}x_{i-1} + Nq_{i-1})/m_{i-1} = q_{i-1}(N - x_{i-1}^2)/m_{i-1} = -q_{i-1}m_i$ ensures that q_{i+1} is an integer. Since m_{i+1} is an integer by the same congruence condition on x_i , p_{i+1} is an integer too. The same kind of argument applied to derive the bound (5) leads to $|m_{i+1}| < 2\sqrt{N}$ and further ensures that $(-1)^j m_{j-1}$, $\sqrt{N} \pm x_i$ are all positive (and so $N - x^2 > 0$ for all i). We have thus, on hand, an infinite sequence of integers (the 'additives') m_0, m_1, m_2, \dots bounded by $2\sqrt{N}$ in absolute value. Hence infinitely many among them must coincide by Dirichlet's box principle. But, as we will see presently, much more is true, namely, (i) there exists an integer s such that $m_{j+s} = m_j$ for $j \geq 1$ and (ii) $m_j = 1$ for an integer j , leading to a solution of equation (2).

In other words, the m_i repeat themselves in a periodical fashion (actually, corresponding to the periodicity of the infinite simple continued fraction expansion for the quadratic irrationality \sqrt{N}).

Before moving on to indicate proofs for assertions (i) and (ii) above, it will be quite in order to quote some interesting observations by Weil (see ref. 1 pp. 23, 24, 94-97, 230-232) on the construction of (p_i, q_i, m_i) . 'The Indian prescription' for the choice of x_i within its congruence class modulo m_i is not quite the one described above, since their rule is 'to make $N - x_i^2$ "small" (i.e. in actual practice, as small as possible), but as the context shows, in absolute value' or in other words, to replace x_i by $y_i := x_i + |m_i|$ if $y_i^2 - N$ turns out to be less than $N - x_i^2$. 'It can be shown that this has merely the effect of abbreviating the procedure somewhat when that is the case,' but though 'numerically useful', can 'make the theoretical discussion much more cumbersome.' Moreover, the above rigorous treatment for constructing (p_i, q_i, m_i) 'may have been known to the Indians only experimentally; there is nothing to indicate whether they had proofs for them, or even for part of them'. 'In order to carry out the cakravālā, a 'starting point' which 'invariably they choose' is the triple $(p_0, 1, m_0)$ 'for which p_0^2 is the closest square to N , above or below.' Finally we are told to iterate the process only till we find an "additive" m with one of the values $\pm 1, \pm 2, \pm 4$ and then to make use of the bhāvanā, i.e. Brahmagupta's procedure for that case. Actually this is no more than a shortcut, since it can be shown that the cakravālā applied in a straightforward manner, would inevitably lead to a triple $(p, q, 1)$ as desired; while this shortcut is quite effective from the point of view of the numerical solution, it destroys the 'cyclic' character of the method, which otherwise would appear from the fact that the additives \dots would repeat themselves periodically corresponding to the periodicity of the continued fraction of \sqrt{N} .

'For the Indians, of course, the effectiveness of "cakravālā" could be no more than an experimental fact, based on their treatment of a great many special cases, some of them of considerable

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complexity and involving (to their delight, no doubt) quite large numbers. Fermat was the first to perceive the need for a general proof and Lagrange the first to publish one. Nevertheless, to have developed the cakravāla and to have applied it successfully to such difficult cases as $N=61$ or $N=67$ had been no mean achievement.'

We now go on to the promised proofs for assertions (i) and (ii) above. The triples (p_i, q_i, m_i) and x_i for $i \geq 0$ as constructed above enable us to obtain an infinite simple continued fraction for \sqrt{N} . Let $\xi_0 := \sqrt{N} + p_0$, $a_0 := 2p_0$, $a_i := (-1)^i(x_{i-1} + x_{i-2})/m_{i-1}$ for $i \geq 1$ and η_j be defined by $\sqrt{N} = x_j + \eta_j$ for $j \geq 0$. Then, in view of the relation $x_{j-1}^2 - N = m_{j-1}m_j$ for $j \geq 0$, we can derive the following:

$$\begin{aligned}\xi_0 &= a_0 + \frac{1}{\xi_1} \text{ with} \\ \xi_1 &:= \frac{1}{\sqrt{N} - p_0} = \frac{\sqrt{N} + p_0}{-m_0} \\ &= \frac{x_0 + \eta_0 + p_0}{-m_0} = \frac{x_0 + x_{-1}}{-m_0} + \frac{\eta_0}{-m_0} \\ \xi_1 &= a_1 + \frac{1}{\xi_2} \text{ with } \xi_2 = -\frac{m_0}{\sqrt{N} - x_0} \\ &= -\frac{m_0(\sqrt{N} + x_0)}{-m_0 m_1} = \frac{x_1 + x_0 + \eta_1}{m_1} \\ \xi_2 &= a_2 + \frac{1}{\xi_3}, \xi_3 = \frac{m_1}{\sqrt{N} - x_1} = \frac{\sqrt{N} + x_1}{-m_2} \\ &= \frac{x_2 + \eta_2 + x_1}{-m_2} = \frac{x_1 + x_2}{-m_2} + \frac{\eta_2}{-m_2} \\ &\dots \\ \xi_i &= \frac{\sqrt{N} + x_{i-2}}{(-1)^i m_{i-1}} = a_i + \frac{1}{\xi_{i+1}} \dots \dots \dots \\ &\dots \dots \dots (7)\end{aligned}$$

Using terminology from the theory of continued fractions², we see that $\xi_0 = \sqrt{N} + [\sqrt{N}]$ has the infinite continued fraction expansion

$$\xi_0 = \langle a_0, a_1, a_2, \dots \rangle,$$

with the natural numbers a_0, a_1, a_2, \dots occurring as partial quotients.

There exists, as we shall see now, a minimal positive integer r such that $a_{i+r} = a_i$ for all i from a certain index l , say. Now $\xi_i = (\sqrt{N} + x_{i-2})/(-1)^i m_{i-1}$ as derived inductively in (7) and coupled with the bound $|m_i| < 2\sqrt{N}$ for all $i \geq 0$, the relation $x_i^2 = N + m_i m_{i+1}$ leads to the

bound $|x_i|^2 < 5N$ for all $i \geq 0$. The number of distinct pairs $(x_{i-1}, (-1)^{i-1} m_i)$ of integers for $i = 1, 2, 3, \dots$ subject to such fixed bounds (depending only on the given non-square natural number N) can only be finite. Thus there exist indices l and $k > l$ such that

$$(x_{l-1}, (-1)^{l-1} m_l) = (x_{k-1}, (-1)^{k-1} m_k), \text{ i.e.}$$

$$\begin{aligned}\xi_l &= \langle a_l, a_{l+1}, \dots, a_{k-1}, \xi_k \rangle \\ &= \xi_k = \langle a_k, a_{k+1}, \dots \rangle,\end{aligned} \quad (8)$$

and therefore

$$\begin{aligned}\xi_0 &= \langle a_0, a_1, \dots, a_{l-1}, a_l, a_{l+1}, \dots, a_{k-1}, \xi_k \rangle \\ &= \langle a_0, a_1, \dots, a_{l-1}, a_l, a_{l+1}, \dots, a_{k-1}, \\ &\quad a_l, a_{l+1}, \dots, a_{k-1}, \dots \rangle \\ &= \langle a_0, a_1, \dots, a_{l-1}, \overline{a_l, a_{l+1}, \dots, a_{k-1}} \rangle\end{aligned}$$

is a periodic continued fraction with period $r := k - l$, proving that $a_{i+r} = a_i$ for all $i \geq l$. Clearly r can be chosen to be minimal.

In the case of $\xi_0 = \sqrt{N} + [\sqrt{N}]$, we can even show that $l = 0$, i.e.

$$\xi_0 = \langle a_0, a_1, a_2, \dots, a_{r-1} \rangle \quad (9)$$

and so is 'purely periodic'. For this purpose, we note first that while $\xi_0 = p_0 + \sqrt{N} > 1$, its 'conjugate' $\xi'_0 := p_0 - \sqrt{N}$ satisfies the inequalities $-1 < \xi'_0 < 0$. Denoting for $\xi_i = (\sqrt{N} + x_{i-2})/(-1)^i m_{i-1}$ its 'conjugate' $(-\sqrt{N} + x_{i-2})/(-1)^i m_{i-1}$ by ξ'_i , we have

$$\xi_i = a_i + 1/\xi_{i+1}, \quad \xi'_i = a_i + 1/\xi'_{i+1}. \quad (10)$$

Now $a_i \geq 1$ for every i , $(-1)^i m_{i-1}$, $\sqrt{N} - x_{i-2}$ are all positive (by construction) and so, $\xi'_i < 0$, leading to $1/\xi'_{i+1} = \xi'_i - a_i < -1$, i.e. $-1 < \xi'_{i+1} < 0$ for every $i \geq -1$. But then, using (10), we have $0 < (-1/\xi'_{i+1}) - a_i < 1$ so that a_i is just the largest integer $[-1/\xi'_{i+1}]$ not exceeding the positive real number $-1/\xi'_{i+1}$. Since, for $k > l$, $\xi_k = \xi_l$ by (8), the 'conjugates' ξ'_k, ξ'_l coincide and so $a_{k-1} = a_{l-1}$. Thus from $\xi_k = \xi_l$ we conclude that $\xi_{l-1} = a_{l-1} + 1/\xi_l = a_{k-1} + 1/\xi_k = \xi_{k-1}$. Iteration yields $\xi_r = \xi_{k-l} = \xi_0$, proving (9).

Now for any $j \geq 1$, ξ_{jr} has the same continued fraction expansion

$$\begin{aligned}\langle a_0, a_1, a_2, \dots, a_{r-1}, a_0, a_1, a_2, \dots, a_{r-1}, \dots \rangle \\ = \langle a_0, a_1, a_2, \dots, a_{r-1} \rangle\end{aligned}$$

as ξ_0 . Hence

$$\frac{\sqrt{N} + x_{jr-2}}{(-1)^{jr} m_{jr-1}} = \xi_{jr} = \xi_0 = \sqrt{N} + p_0,$$

$\sqrt{N} (1 - (-1)^{jr} m_{jr-1}) = (-1)^{jr} m_{jr-1} p_0 - x_{jr-2}$. But if $c + d\sqrt{N} = 0$ for integers c, d , then necessarily $c = d = 0$. Hence, in particular,

$$(-1)^{jr} m_{jr-1} = 1.$$

Whenever jr is even (e.g. $j = 2$ for odd r and $j = 1$ for even integers r), we have $m_{jr-1} = 1$ and the triple $(p_{jr-1}, q_{jr-1}; 1)$ gives a solution of the diophantine equation (2) proving assertion (ii). Assertion (i) also follows from above easily.

An example of how unsparing a critical analysis can tend to be (when intended or called for) may be found in Weil's remarks on Euler's contribution to the topic of 'Pell's equation' and the related continued fraction algorithm, on pages 232-233 (ref. 1): 'while Euler drew attention' to the periodicity and 'palindromic' property of the partial quotients a_i in the 'continued fractions for square roots \sqrt{N} , as well as to their use in solving Pell's equation, there is no sign that he (Euler) ever sought to back up his findings by anything more than experimental evidence. He (Euler) did mention that the values obtained by his process for the integers B_i, A_i, m_i are necessarily bounded...; from this he (Euler) could at least have derived the conclusion that the sequence (m_i) is periodic from a certain point onwards, but he failed to mention this, or did not bother to do so'. When in his later years (after Lagrange gave a 'definitive treatment' of the subject, based on the continued fraction algorithm...), 'Euler came back to the topic of Pell's equation, he added nothing of substance to what by that time was already public knowledge on that subject'.

Fermat must have been in the dark about the contribution of the Indian mathematicians to the solution of (2) and also possibly about Archimedes' *Problema bovinum*. He offered (in 1657) the problem of solving equation (2) (in integers, of course!) as a challenge to the English mathematicians and all others. In a personal letter to Huygens, a few months later, commenting on the solution by Wallis and Brouncker, he observed that 'the English had failed to give a general proof'; such a ('general') proof, according to Fermat, could only be 'obtained by descent'. But perhaps 'Fermat's method of solution (for (2)) did not greatly differ

from the one he got from Wallis and Brouncker' and 'he had been able to extract from it a formal proof of the fact that it always leads to a solution'. The method 'which Wallis credits to Brouncker' is 'equivalent to the Indian cakravāla method as well as to the mod-

ern treatments based on continued fraction'.

1. Weil, André, *Number Theory – An Approach Through History – From Hammurapi to Legendre*, Birkhäuser, Boston, 1983.

2. Niven, I. and Zuckerman, H. S., *An Introduction to the Theory of Numbers*, Wiley Eastern, New Delhi, 1976.

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Addendum

Fifty years of the exact solution of the two-dimensional Ising model by Onsager

Somendra M. Bhattacharjee and
Avinash Khare

[*Curr. Sci.*, 1995, **69**, 816–821]

We would like to make the following corrections/additions which we learnt after the paper was written.

W. Lenz was working in Rostock University (not Rostalk as mentioned in the text).

E. Ising was a teacher (and later became the headmaster) of a Jewish school in Germany from 1934 to November 1938. When his school was damaged, he managed to leave Germany. It was as late as 1949 that he realized that his name had become famous.

It seems that Ising also agrees that the model should be named the Lenz–Ising model. The name 'Ising model' became popular following the title of Peierls' paper¹².

It appears that Heisenberg in his 1928 paper⁸ also thought that at least eight nearest neighbours are needed for a phase transition. We are not sure whether this comment by Heisenberg refers to the Ising model or to the new model he proposed in that paper.

We thank Prof. Sigismund Kobe of University of Dresden for several clarification.

MEETINGS/SYMPOSIA/SEMINARS

COAL-96 International Symposium on Coal: Science, Technology, Industry, Business and Environment

Date: 18-19 November 1996

Place: Dhanbad

The symposium is being organized to commemorate the Golden Jubilee of Central Fuel Research Institute. Topics include: Coal science, Advanced coal preparation, Advanced coal utilization, Coal chemicals.

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1996 Asia-Pacific Microwave Conference — APMC '96

Date: 17-20 December 1996

Place: New Delhi

Topics include: Any paper concerned with the utilization and application of microwave theory will be considered.

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DAE Solid State Physics Symposium

Date: 27-31 December 1996

Place: Mumbai

Topics include: Phonon physics; Electron states and electronic properties; Magnetism and magnetic properties; Semiconductor physics; Physics of defects and disordered materials; Transport properties; Superconductivity and superfluidity; Liquids, liquid crystals and plastic crystals; Phase transitions and critical phenomena; Surface and interface physics; Non-equilibrium phenomena in solids; Physics of complex systems; Resonance studies and relaxation phenomena; Solid state devices, techniques and instrumentation.

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International Seminar on Quaternary Sea-level Variation, Shoreline Displacement and Coastal Environment

Date: 20-26 January 1997

Place: Thanjavur

Topics include: Inter-glacial sea levels and coastal environmental changes; The record of sea-level changes on continental shelves; Coral reefs and sea-level change; Sediment sources and sinks in coastal zones, particularly sand dunes and estuaries during the Holocene; Coastal changes associated with seismic events; Storm signatures and El Nino events and their impacts on coasts; Exploration, development, refinement and application of new techniques to elucidate the understanding of coastal dynamics.

Contact: Prof. G. Victor Rajamanickam
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4th National Seminar on Physics and Technology of Sensors

Date: 3-5 February 1997

Place: Vallabh Vidyanagar

Topics include: Sensor fundamentals and physics; Sensor materials and their processing; Fabrication techniques; Micromachining and integral sensors; Sensor technology; System and applications; Chemical sensors; Gas and pollution related sensors; Mass sensitive devices; Intelligent smart systems; Industrially important sensors; Agro-based sensors, Automobile sensors, Bio-sensors and biomedical sensors; Interface electronics; Physical sensors: Thermal, magnetic, CNC sensors; Optical fibre-based sensors.

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3rd International Symposium on Innovations in Pharmaceutical Sciences & Technology

Date: 7-9 February 1997

Place: Ahmedabad

Programme summary: I. Plenary and invited lectures (Pharmaceutical technology and novel drug delivery systems, Drug discovery and design, Pharmacokinetics, drug metabolism and clinical pharmacology and Traditional medicine and medicinal plant products). II. Pre-symposium workshops on Oral Controlled Drug Delivery and Drug Discovery – Chemical Approaches. III. Poster

presentations.

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National Conference on Spectrophysics (NCONS 97)

Date: 10-12 February 1997
Place: Chennai

Topics include: Raman spectroscopy; Infrared spectroscopy; Vibrational analysis and molecular structure; Band shapes, dynamics; Band intensities; Macromolecules and Biological systems; Surface and Interfacial phenomenon, SERS; Inorganic materials, matrices; Semiconductors and semiconductor micro structures; Superconductors; Phase transitions; Effects of temperature and pressure; Industrial and medical applications; Pollution analysis; New techniques; Characterization of crystalline & ceramic compounds; Nuclear spectroscopy; Hyperfine structure studies; Laser and laser-based spectroscopy.

Contact: Dr S. Gunasekaran
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Seminar on Herbal and Microbial Pesticides

Date: 14-16 February 1997
Place: Varanasi

Technical sessions include: 1. Old traditional and indigenous pesticides, 2. Herbal pesticides, 3. Microbial pesticides.

Contact: Dr N. K. Dubey
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Training Course in Palaeoseismology

Date: 28 March-6 April 1997
Place: Dehra Dun

A training course is being organized for young scientists/ research workers actively involved in studies on seismology, seismotectonics, active tectonics, seismic hazard zonation and related fields involving extensive theory and field work including study of trenches for palaeoseismology. The faculty will include experts from USA, Japan and India.

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Second Indian National Conference on Harbour and Ocean Engineering (Inchoe-97)

Date: 8-10 December 1997
Place: Thiruvananthapuram

Themes include: Marine hydrodynamics, Port engineering and management, Marine structures and materials, Dredging and environmental aspects, Monitoring and instrumentation, Coastal processes and coastal zone management.

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GOVERNMENT OF INDIA

DEPARTMENT OF SCIENCE & TECHNOLOGY

Programme on Nonlinear Phenomena, Complex Matter and Biologically Inspired Physics

The importance of nonlinear science, soft condensed matter (such as colloids, powders, polymers and membranes) and biologically inspired physics has emerged very clearly in recent years. In view of this and given the large mutual overlaps of these disciplines as well as their interdisciplinary nature, the DST has considered it fit to constitute a separate Programme Advisory Committee (PAC) specifically to foster research in these areas. Suitable project proposals are invited in the above general fields.

Research proposals should pay particular attention to the development and application of ideas and techniques that cut across disciplines and/or seek to elucidate and provide insight into the general paradigms of complexity and nonlinearity.

Proposals that come under this general purview include:

Dynamical phenomena far from equilibrium, driven systems, chaos, especially spatiotemporal. This encompasses both model systems such as sandpiles and driven lattice gases as well as 'real' examples such as colloids in shear flow and sedimentation, turbulence, kinetics of phase ordering, granular matter (powders), earthquakes, pattern formation, etc.

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Biologically inspired physics. Models in evolutionary biology and population genetics, evolution of complexity, neural networks, optimization, protein folding, motor proteins and molecular ratchets, etc.

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CURRENT SCIENCE

A fortnightly journal of research
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1. Mukundan, T. and Kishore, K., *Curr. Sci.*, 1991, **60**, 355–362.
2. Constantine, G., in *Biology of Bats* (ed. Wimsatt, W. A), Academic Press, New York. 1970. vol 1, pp. 319–322.

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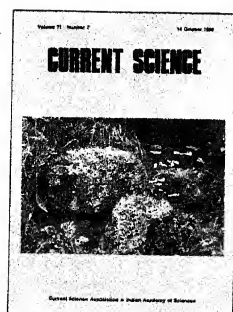
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COVER. *Stereocaulon* species growing in association with mosses on rocks at 3300 m in Nanda Devi Biosphere Reserve. See page 568.

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In this issue

Weather forecasting

The evolution of meteorology in general and weather forecasting in particular to its present status as an objective scientific discipline has been a fascinating and tortuous journey. The use of complex mathematical models for dynamics of weather and climate, and their numerical integration occupies the central place in today's science of weather and climate prediction. This numerical weather (or climate) prediction, however, requires involvement of a number of seemingly unconnected disciplines. The progress made in the last few decades would not have been possible but for developments in physical and mathematical modelling, numerical methods, high performance computing and visualization, data management and analysis techniques and observing systems. In this issue Hunt (**page 520**) provides a succinct and captivating account of the current status, problems and future directions of the science of weather and climate prediction. The article provides a non-technical and encompassing survey of various methods currently in use for prediction and those which hold promise, such as expert systems, hybrid methods (man-machine mix) and neural networks. The article also provides a lucid account of emerging concepts and various problems that make the science of weather and climate prediction an exciting scientific challenge.

P. Goswami

Lichens of the Nanda Devi Biosphere Reserve

More often seen as grayish-greenish patches on tree-trunks, on stone walls of old buildings, or even on bare rocks, the lichen seem to be

one of the least glamorous (and therefore most neglected) group of living organisms. However, they are one of most widespread group of plants, with many unique and fascinating characteristics.

A lichen is in some sense a single (but symbiotic) organism, made up of two distinct partners – who by themselves are capable of leading an independent life; the dominant partner is a fungus and the subordinate, photosynthetic partner is an alga (though sometimes cyanobacteria take its place). Lichen can survive (as far as their natural history is concerned, on a global scale) in extremely inhospitable habitats and climates – from high latitudes (close to the poles) to hot, dry deserts. Paradoxically, at a local scale, they are very susceptible to adverse environmental conditions such as pollution, since they readily absorb pollutants dissolved in rainwater and dew. In fact, they are extensively used as bioindicators of pollution – including that caused by radioactive fallouts. Lichens grow very slowly – 10 to 25 millimeters in 100 years is the rate quoted in alpine and arctic regions. They are also very long-lived, surviving for more than a thousand years. In fact, the age of a specimen from Swedish Lapland has been estimated to be around of 9000 years old, making it one of the oldest living organisms.

About 25000 species of lichens have been described from all over the world. However, this is one of the least actively studied groups. An authoritative text-book published in 1967 estimated that it would take about 20 years for lichen to be as well described as fungi – and the 1983 edition of the same book has discreetly changed the phrase '20 years' to 'many years'.

From India, studies on nearly

2000 species of lichen and almost 700 species of macrolichen have been reported, mostly dealing with taxonomic aspects. One of the first investigations focusing on the community ecology of lichens is reported on **page 568** of this issue by Hans Raj Negi and Madhav Gadgil. Covering the altitudinal range from 2100 meters to 4500 meters in the western part of the Nanda Devi Biosphere Reserve, and a diversity of habitats ranging from roadside grass and scrub to pine forests and alpine meadows, the authors have recorded 76 species of macro-lichens from 16 transects (each 50 m × 10 m).

Unfortunately, just like the more high profile mammal and bird species, the lowly lichens too appear to be under threat from human interference such as deforestation. More perceptively, the authors have identified a more subtle threat from the overgrowing weeds. These weeds, which rapidly deplete nutrients from the soils, have become more common due to a ban on grazing in the meadows – ironically introduced for protecting the biodiversity in the meadows! The authors also point out how the lichens, due to their essential oils and nitrogen-fixing capabilities, could play an important economic role in that region.

N. V. Joshi

'Mariner' element

Getting a free ride is second nature to humans, but this trait is often combined with good sense. On a Kanpur-Jhansi train journey some years ago, I asked my neighbour why, without reservations or even tickets, he and his fifty odd friends had entered a reserved compartment and made themselves com-

fortable. He looked with pity at my stupidity and replied that the reserved compartments were the only ones with space for them. Transposable elements are like humans taking a free trip. These elements, bits of DNA present in an organism can often hop around the host's chromosomes, often 'unnoticed' but sometimes causing serious trouble. Are these elements parasites? Where did they come from, how many of them can an organism tolerate? While many biologists delve into these basic questions, others have used transposable elements as tools for introducing foreign DNA into organism. Transposable elements have characteristic sequences at each of their termini which are required for integration and mobility in the host genome. In addition, these elements encode protein products, 'transposase' that are required for effecting their movement in the host's genome. In a major breakthrough in biology, Gerry Rubin and Allan Spradling, in 1982, manipulated transposable elements in the fruit fly *Drosophila* so that it could be used for the insertion of cloned genes into the germ-line of flies. They inserted these cloned genes in between the ends of a transposable element and injected this modified DNA into the cells of the egg that give rise to gonads of the fly. Adults that de-

veloped from these injected eggs carried the modified transposable element inserted in their 'germ-cells' and this integrated DNA could be detected in the next generation. Rubin and Allan Spradling's experiments transformed *Drosophila* biology from a study of an obscure fly by obscure humans to an area of immense general relevance. Since their pioneering work, scientists have been able to study the regulation of genes and gene products during animal development by putting genes back into flies. They have shown that the structure and function of many gene products are conserved during evolution. It seems that flies and human may actually be very similar in many aspects of their development, something that was not obvious at all.

While inserting DNA into fruit flies now appears trivial, similar transformation of other insects remains a challenge. Despite several attempts, insects such as mosquitoes and silk-worms remain refractive to transformation. Part of the reason for this has been that these organisms have been studied, genetically, far less than *Drosophila*. Another reason is the rather naive, in hindsight, assumption that transposable elements isolated in *Drosophila* will function in a similar manner in all insects. Initial

attempts with *Drosophila* elements failed, for reasons that are only now becoming clear. The analysis by Hugh Robertson and colleagues of the Mariner transposable was a major positive step. The Mariner element is present in a very large variety of insects and appears to have been acquired by these insects relatively recently during evolution. Their recent appearance and spread make them a good general candidate as a vehicle for insect transformation. One of the insects of great commercial importance is the silkworm moth *Bombyx mori*. Initial attempts to assay for the presence of the Mariner element in this moth proved negative. However, recent experiments, reported by Mathavan *et al.* (page 577) demonstrate a Mariner-like element in silkworm. This suggests that Mariner can invade this organism and perhaps be used as a vehicle for transformation. There is a long way to go before germline transformation becomes feasible and of general use in silkworm. However, intense work on Mariner and other transposable elements and tests of their ability to integrate into the germline chromosomes may make commercial exploitation of this insect by introducing genes of interest a distinct possibility.

K. VijayRaghavan

CURRENT SCIENCE

Volume 71 Number 7

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CORRESPONDENCE

Science education for the poor

The need for broadening the Science base in India, in order to tap talent from the entire population of the country so as to make the Indian scientists more competitive in the international arena has been emphasized (*Curr. Sci.*, 1995, **69**, 287). It is heartening to learn (*The Hindu*, August 21 and 29, 1996) that the Government proposes to start Residential Schools in 100 districts in the country in order to impart quality education to the poorest of the poor. This is very much required as there is little hope for poor children (who come through the midday meal programme) to become doctors, engineers and scientists if the current trend of commercialization of education continues.

The criteria for admissions to these Residential Schools should be different. The selectors should go to the elementary schools run in the respective areas to select the best students among the poorest of the poor. Remuneration of teachers in these new Residential Schools should be much higher than for those in other

schools and they should not be allowed to give private tuition, which destroys the education system in this country. On the other hand, incentives should be provided for teachers who work hard to get the best out of their students. In addition, parents should be supported financially so that they do not take away their wards half way through.

I would urge the Government further to start 'National Science Universities' also for the poorest of the poor. One such 'elitist' university (on the IIT, IISc model) in each state, exclusively for the best among the students who come from the above mentioned Residential Schools, will be required to make them doctors, engineers and scientists. Again, remuneration of teachers of these National Science Universities should be much higher than those in other Universities so that the best will come forward to serve the poorest of the poor. Admissions to these National Science Universities at the research level can be made open to the best among all Indians. I believe that

these measures, in addition to helping the poor, will also make Indian science highly competitive.

There is a growing feeling among the common public and the political leadership that the higher education and scientific research are toys of the rich. This is one of the reasons, for the present reluctance of Government to spend scarce resources on higher education. If the science leadership and the academies come forward to help the poorest of the poor, no Government will ignore higher education and science. I strongly believe that we still have leaders among us who will appreciate and come forward to implement these ideas as they will not like to miss a chance to become the 'messiahs' of the poor.

M. PERIASAMY

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RESEARCH NEWS

Vitamin A analogues and cancer

P. C. Bhattacharyya

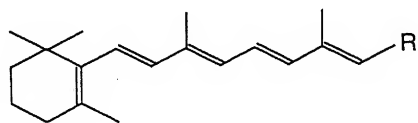
Vitamin A is known for its role in general growth and healthy eyes. Its deficiency in diet results in night blindness. Vitamin A is essential to fight against infectious diseases such as diseases of respiratory system and diarrhoea. In nature, it occurs in two forms, retinal and dehydroretinol. Retinol is vitamin A and retinal, an

aldehyde of vitamin A, is derived from vitamin A by oxidation. The retinal differs in having one or more conjugated double bond and has approximately one-third the biological activity of retinol, i.e. vitamin A.

Majority of human cancers arise in epithelial tissues of lungs, bladder, breast,

colon, pancreas, stomach, uterus, prostate, etc. Retinoids which include retinol, retinal, retinoic acid, etc. are implicated in the maintenance of normal epithelial cell differentiation and are useful for the treatment of certain kinds of cancer, e.g. vitamin A can cure mouth cancer. Since carcinogenesis is characterized by abnor-

RESEARCH NEWS



Retinol : R = CH₂OH
Retinal : R = CHO
Retinoic acid : R = COOH

mal cell differentiation, the primary need will be to use agents that can bring about normal cell differentiation. Retenoids, especially the naturals such as retinyl esters and others, can help in the prevention of cancer.

Lack of promising results by retinoids may be because these do not always reach potential sites in adequate concentrations. Because of fear of excessive storage of retinyl esters that may cause damage to the liver, there may be a failure in administration of appropriate doses. The all-*trans*-retinoic acid (ATRA), another retinoid, is used in the treatment of skin cancer and leukaemia, especially acute promyelocytic leukaemia (APL)¹. Indeed, this presents a breakthrough,

although 13-*cis*-retinoic acid has been found to have a marked inhibitory effect on the development of both preneoplastic and neoplastic lesions of the bladder. Consequently, retinoids, the structural analogues of vitamin A represent a new class of compounds with remarkable therapeutic activities. It remains for the organic chemists to synthesize further structural variations of retinoid skeletons.

Recent studies² showed interesting results on the application of supplements of vitamin A and zinc. These give protection against stomach cancer. Studies conducted in China showed that the supplement can act as the anti-cancer agent. Philip Taylor, Chief of the Cancer Prevention Studies at the US National Cancer Institute in Bethesda, Maryland, confirmed usefulness of these supplements. The research study organized by Chinese and American researchers prescribed daily intake of one of the four combinations such as vitamin A and zinc; B vitamins such as riboflavin and niacin; vitamin C and molybdenum; and an anti-oxidant especially the cocktail of β -carotene (precursor of vitamin A), vitamin E and

selenium. These researchers believe that vitamin A prevents cancer because it stops cell differentiation processing to a minimum and zinc added to vitamin A accelerates the cellular transport of vitamin A. However, vitamin A and zinc formulation is assumed to be the best. According to these workers, cancers of stomach can be reduced by two thirds.

The Harbin team found that arsenic compound in traditional Chinese medicine helped a complete remission in more than 70% of APL patients. Zhung reported³ that the side-effects were almost nil and that if there is any toxic effect, it can greatly be minimized by intravenous administration of the drug. It is believed that arsenic trioxide works differently with ATRA. The mechanism awaits elucidation.

1. Mervis, Jeffrey, *Science*, 1996, 273, 578.
2. Kolberg, Rubeca, *New Sci.*, 1993, 22 May, 7.
3. Ting-Dong, Zhung, *Science*, 1995, 17 Nov. 1144.

P. C. Bhattacharyya, North Station Road,
P.O. Agarpara 743 177, India.

Research Snippets (edited by K. Manjula)

A feast for the pharaoh

The art of producing beer and wines is said to be almost eight thousand years old. Ancient methods of preparation chiefly relied on exposing fruit juice and cereal extracts to air. Reports of the use of fermentation techniques during wine-making are said to date back to four thousand years ago in the Egyptian and the Babylonian civilizations. Artistic descriptions indicate that bread and beer were the staple food of the Egyptian civilization. Earlier documented evidence of this ancient culture indicates that coarsely milled wheat used for making bread was in fact well-kneaded but full of chaff and grit. Baked bread was sometimes crumbled and strained through sieves into vats with water to initiate the process of making beer (*Biotechnology of Malting and Brewing*, by J. S. Hough).

More direct evidence was further

obtained from remains recovered from tombs and settlements dating throughout the Pharaonic period. Examination of such remains would provide reasonably authentic evidence as the Egyptian climate allowed for good preservation of the starch content of the food specimen. Earlier studies relied on the use of light microscopy of samples such as the pots wherein liquor was stored as well as food packed into Egyptian mummies for the next life. Results were often obscure and unreliable due to a poor resolution of the starch granules. A study conducted by Delwen Samuel (*Science* vol. 273, 26 July 1996) has interesting facts about how the ancient Egyptians baked their bread and brewed their beer. The study relied on the use of Scanning Electron Microscopy in order to systematically analyse desiccated loaves and cereal residues obtained from a site where Egyptian workmen built tombs in the Valley of Kings and Queens. The focus

of attention was analysing the physical properties of starch granules that are known to vary according to the method used during the food processing. When starch (a polymer of glucose units linked together through glycosidic bonds) is heated in water, the starch granules swell, fold and merge. Depending on the water content of the food sample, the starch granules either remain discrete or fuse together creating hazy boundaries (as revealed through an SEM). The study has revealed that the microstructure of Egyptian bread resembled that of the modern bread with some loaves even leavened by the use of yeast. Contrary to the older belief, brewing was done not by a crude method but was in actuality a two-step process. Heated and malted cereal grains were mixed with unheated and sprouted grains, decanted and fermented to produce Egyptian beer. Cleopatra obviously wined and dined her guests in style!

Finicky flights of fancy

With the curtains drawn on the recent Olympic Games held in Atlanta, a question that seems to haunt the mind of many a physiologist is how far the Olympic motto can actually be stretched. How high, how swift and how strong can today's athlete become? Ehsan Masood wonders (in a briefing in *Nature*, vol. 382, 4th July 1996) how far this 'record-breaking' could last. Roger Woledge, professor of physiology from the University College, London believes that where the performance of the human body is concerned, there is certainly a limit. After all, the human race cannot be likened to an in-bred racehorse. A recent study conducted at University of California compared the metabolic rates of 37 species including human beings. In most cases, the ratio of the metabolic rates (that actually indicate the energy level) between subjects in the sleep phase and the active phase did not exceed 7:1. Even when unlimited food was provided to the subjects, this rate did not vary significantly. In retrospect, while improved training, sports equipment, clothing and nutrition may indeed enhance an athlete's performance, no one can really go on and on. There seems to be a well-meaning dot that punctuates every hundred metre dash.

'Y' are we so hungry?

Mechanisms that balance the food intake and the energy expenditure can determine whether an individual is fat or thin. A couple of years ago Jeffrey Friedman, from the Howard Hughes Medical Institute, identified the 'obese gene' from mice, whose protein product actually regulates this balance. The counterpart of this gene in the humans codes for a protein called 'leptin' that can suppress the appetite of an individual and increase the metabolic rate. Later, Millenium Pharmaceuticals succeeded in identifying the corresponding receptor situated on the cell membrane

that binds to the leptin molecules. The protein entering the cell in this manner would then proceed to execute a particular metabolic function. Any aberration either in the obese gene or in the gene coding for its receptor protein is said to result in obesity. Even while Millenium Pharmaceuticals is involved in the formidable task of designing drugs to control obesity, the attention has now been diverted to a neuropeptide that seems to possess a master control of the feeding habits of an individual.

A few years ago, peptide hormones were in the news because of the crucial functions they are able to execute in the human body. PYY, one such hormone that is secreted by the pancreatic cells, has a unique amide attached to its C-terminal tyrosine group. This was later found to bear an uncanny resemblance to the Neuropeptide Y (NPY) that is widely distributed in the limbic regions of the brain. Earlier results have indicated that NPY binds to specific receptors of the 'Y' class. This class of receptors (Y1, Y2, and Y3) is found in mammals and can bind to such peptides with varying affinities resulting in the triggering of a biochemical signalling pathway.

Evidence so far indicates that the signal triggered by the NPY-receptor binding regulates the feeding habits of the individual. Attempts at identifying the receptor have finally yielded results, according to a recent report in *Nature* (Gerald *et al.*, vol. 382, 11th July 1996). The total mRNA (which acts as an intermediate in the DNA-to-protein transition) isolated from the hypothalamus of a rat was converted into a complementary DNA (cDNA) library using conventional methods in genetic engineering. The cDNA molecules were later introduced into an expression cloning system, where conditions allow for the DNA to be decoded into its corresponding protein. When the protein products were exposed to labelled NPY molecules, the protein that bound chemically to the labelled protein was fished out and its properties studied. This protein (that is actually the receptor) is said to bear scant resemblance (as judged by its amino acid sequence analysis) to the

older receptor molecules, and has been duly named the 'Y5' receptor. In humans, the Y1 receptor identified earlier also bound to the NPY peptide. So in what way is the new Y5 receptor (that has also been identified in humans) related to Y1? For some unknown reason, the genes coding for the Y1 and the Y5 receptors map to the same locus of the same chromosome but in the opposite orientation.

Using the rat as the model system, an elaborate study of the Y5 receptor was conducted starting with the localization of this receptor in various tissues of the brain. Evidence now points out that through an intricate circuitry created by the receptor location in the brain, NPY may also regulate the emotional aspect of feeding behaviour. Y5 is clearly the best candidate for studying and understanding the physiology and psychology of feeding behaviour and that includes several feeding disorders.

The 'not so sweet news'

Aspartame is a high intensity artificial sweetener which is being marketed under various brand names like 'Equal', 'NutraSweet' and 'Spoonful'. A recent report in the Journal of Diabetic Association of India' (Vol. 35) cautions the indiscriminate use of this sweetener that can lead to as many as 90 different documented symptoms including headaches, visual problems, and even heart palpitations. Aspartame, (which is L-Aspartyl Phenylalanine Methyl Ester) can breakdown in the body into amino acids and methanol. As there is no ethanol in Aspartame to counter the ill-effects of Methanol, it can lead to several of these undesirable side-effects. The FDA, however, claims that a daily dose up to 50 mg/kg body weight is safe.

India – Economic liberalization and biomedical and health sciences research*

V. Ramalingaswami

A cliché 'Think Globally and Act Locally' is doing the rounds in the corridors of the World Health Organization in Geneva. It reflects the spirit of discussions at this symposium on 'Indian science after liberalization'.

The spirit of liberalization

The 'fact sheet' distributed at the meeting lists a series of measures that the Government of India took in 1991 to 'unshackle the Indian industrial economy from the cobwebs of unnecessary bureaucratic control'. It lists a number of measures such as abolition of industrial licensing, direct foreign investment up to 51% foreign equity, entry of private sector in areas hitherto reserved for public sector, etc., but as Mashelkar said earlier, the true spirit of liberalization is the important thing.

Performance indicators

An objective discussion of the issues under the rubric of Indian science after liberalization is possible only when there are objective indices of performance in various sectors. Such indices are not easy to come by as yet and so a discussion in depth on this topic is not yet entirely feasible. One has to indulge largely in a qualitative reflection.

Basic science indicators

As Herman Bondy said, basic science is the well-spring of modern technology. One yardstick of measuring the impact of liberalization on Indian science is to see to what extent creativity and advances in basic sciences had been stimu-

*Based on comments made at the Indian Science Writers Association – 4th National Convention – in a symposium entitled, 'Indian Science After Liberalization' at New Delhi, on the 3rd May 1996.

lated as precursors of innovative technologies. The whole field of transforming findings in basic science to meaningful application in resolving health problems affecting vast numbers of people needs attention¹.

There is evidence that India's capabilities in basic biological science research in fields such as molecular biology, molecular genetics, immunology and cell biology are now pretty well developed. The scene is set for exploitation of this capability for economic growth through a biotechnological revolution subserving medical and health care².

The concept of partnership with industry is articulated beautifully in the Council of Scientific and Industrial Research document: *CSIR 2001 – Vision and Strategy*³. It is not enough to liberalize the economy through the series of measures promulgated by Government. The atmosphere must be conducive to the effective implementation of those measures and to bring about active collaboration between science and industry. This has been the Achille's heel of Indian science. However, there are signs that basic discovery and application are moving closer together in India. Even so, the sight of so many biotechnological diagnostic products developed in Indian laboratories lying unabsorbed into the health care system in a major way is all too common. This is an area, I would suggest, needs most urgent dialogue between science, industry, government, representatives of the health care system and the public.

Product indicators

Science has to be 'manipulated' into processes, products and devices and this can be quantitated to measure performance. The journey from a laboratory-based technology to the market place is the most crucial one for the Indian sci-

entific enterprise as alluded to already. It is an enigma to friends of India as to why India had been unable to jump on to the band-wagon of market economy despite the presence of a very significant science and technology infrastructure. The situation in this regard may be beginning to change.

Products and patents are the hallmarks of today's market economics. I would broadly endorse Mashelkar's comment: 'Patent before you publish'. It is like saying 'look before you leap'. It is time to de-emphasize the slogan 'publish or perish' but publication of one's work in peer-reviewed journals and its presentation in scientific fora must continue to be the general rule within these guidelines in today's world.

Social impact indicators

If science is to be the foundation of economic progress as well as social well-being, social impact indicators, although they may take time to reveal themselves, would be legitimate criteria to look for the impact of liberalization on Indian science. One obvious reflection would be to look at the poverty line, whether it is moving up or down as a function of time. The debates on how much poverty there is in India tend to become abstruse⁴. But, on the whole, whether it is government's or academicians', the data indicate a decline in the poverty figures, although understandably, in the case of government figures, the data are more impressive – a decline in poverty figure from 25.5% of the population in 1987–88 to 18.9% in 1993–94 (ref. 4).

There are other indicators such as infant mortality, life expectancy, employment, IQs and growth of children, environmental quality, and indicators pertaining to the well-being of women. Data on these are badly needed but instant changes should not be expected.

Joint ventures

There are apprehensions that opening up Indian S&T enterprise to foreign collaboration might erode into the dearly-held Indian policy of self-reliance. It must be remembered that in entering into joint ventures between Indian and foreign industry one has to negotiate from a position of strength. True partnership seeks to leverage complementary strengths and overcome weaknesses of individual partners⁵.

Privatization, disease palaces and the face of liberalization

To the ordinary citizen, the impact of liberalization on medical science in India is the overnight erection of large tertiary care institutions in urban areas. These may serve a useful purpose in making high quality care available largely to those who can afford the costs involved. As it is often claimed, they may also liberate the public hospitals from expropriation of their services by the privileged sections of society and enable more services from these hospitals to flow to the low-income groups. Large tertiary care institutions in the private sector are required by law by the state governments to make available a proportion of their caring services free of charge to those who cannot afford them. How this formula works in meeting the health care needs of the poor, even in a small and significant way, is a matter for further study. If it is largely serving as a conscience pacifier, how can this powerful force for health be modified and channelized to attain better equity in services? I know of several physicians working behind the high walls of these institutions who are looking for approaches by which these institutions could be linked to public sector health care institutions to provide back-up support, thus becoming integral parts of mainstream-caring services to the people. In countries like India which have a dual system, in reality a pluralistic patchwork of services, the subtle connections between public and private sectors in health care need careful analysis. The social and economic im-

pact of less restrained use of technology in treating individual patients needs to be evaluated⁶. The practice of medicine today is filled with an aura of high technology, expensive medical procedures, excessive laboratory investigations and the high drama of life-saving interventions. It creates stresses on resources, especially acute in low-income countries. How to cope with these stresses in such countries, particularly with the push of privatized health care and how to maintain a balance with considerations of equity in mind, constitutes one of the major challenges of economic liberalization in resource-poor countries.

From a population health standpoint, it is the effect of any reform on primary health care that is of utmost significance. Equally, how the reform addresses the issues of supportive referral services from primary to secondary and tertiary care level needs to be ascertained and mid-course corrections applied.

Plant-derived therapeutic substances

The directing of chemistry of natural products to the relief of human ailments and maintenance of human health is another aspect of the liberalization – health care equation. Intensive research for new therapeutic substances from natural sources is required – a process which has already begun. The discovery of bio-active natural products from Indian medicinal plants is an area of considerable significance for the health care of India's teeming millions. In line with the great legacy of India in this regard, much effort is being expended in Indian research laboratories in pursuit of this goal. Some progress has been made in this direction and several speakers have already alluded to this. This could be yet another indicator of liberalization and health sciences. There are immense opportunities for the discovery of new natural products against challenging diseases for which either the existing therapeutic substances are ineffective *ab initio* or the microbial pathogens transformed from sensitive to resistant strains. It is quite conceivable that plant

genes could be discovered that code for novel molecules of preventive or therapeutic or immunomodulatory value.

Conclusion

In a discussion on science and technology and the economy, there is a need for objective data on the trends in R&D expenditure, the areas of basic research that impact on the economy and in the case of biomedical and health sciences, advances in medical sciences and biotechnology and their impact on health care of people, not ignoring equity issues⁷.

It would be appropriate to conclude with the observations made by Douglas Black, past President of Royal College of Physicians, London, who said of market economies: 'There is an overconfidence in the beneficence of the market and an underawareness of the difference between a business and a service. Is managerial efficiency more important than the skills and dedications of doctors?'⁸.

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India needs a National Biodiversity Conservation Board

T. N. Khoshoo

India's stakes in biodiversity are very high not only because it is a country rich in biodiversity but, out of the twelve centres, it is also a very important centre of origin of agri-biodiversity. Furthermore, the country as a whole is predominantly biomass-based with largely bio-industrial pattern of development in an unusually large number of villages, where over 76% of its population lives. The country is also a signatory to the Biodiversity Convention (1992), but such a decision was taken without any serious consultation with the scientific community. This has resulted in India landing itself into a situation where policy on biodiversity has overtaken the underlying science and technology. A major issue is how well scientific and technical knowledge can be harnessed into public policy. This paper summarizes India's strengths and weaknesses in this area and urges that the Government of India appoint a small scientifically and technically sound National Biodiversity Conservation Board. This would not only enable to prepare a cadre of conservation biologists, but also help to generate products and give the requisite bargaining power to the country in the international arena during negotiations.

In common parlance, biodiversity may be defined as species richness (plants, animals and microorganisms) in a given habitat be it on land, in fresh water or sea, or as parasites or symbionts. Biodiversity is critical to the very health and stability of the biosphere and renewability of biomass, soil, water and air, together with oxygen, carbon, nitrogen and phosphorous cycles. Thus biodiversity renders free recycling and purification services together with natural pest control.

A subset of biodiversity is *genetic diversity* which occurs in the form of interbreeding *populations* of a given species. Populations of different plants, animals and microorganisms in a given habitat, existing as an interacting system, are known as *communities*. An aggregate of communities occurring as an interacting system in a given ecological niche makes an *ecosystem*. Different ecosystems in an ecological region occurring as an interacting system constitute a *biogeographical province*. All biogeographical provinces in a major ecological zone constitute a *realm*, and all realms on the surface of the earth together constitute the *biosphere*—the living mantle around the world occurring on land, in fresh water and in sea. Biodiversity is also the source of all living materials used as food, shelter, clothing, biomass energy, medicaments, etc. and host of other raw materials used in bioindustrial development. These along with metallic and non-metallic minerals constitute the basic wealth of any country. Ultimately, economy and ecology of a country depend on the health of these resources.

There is increasing pressure on natural habitats due to growing human population and enhanced pace of socio-economic development. This has led to the degradation of parts of earth's biosphere, and has resulted in loss of biodiversity and agricultural productivity. Such losses of species *are for ever* and affect not only plants, animals and microorganisms in nature together with those under cultivation/domestication and used in industry, but also those whose value has yet to be ascertained.

The evolutionary history of earth is replete with examples of both extinction of old and origin of new species taking place simultaneously. Infact, geological times have witnessed five major episodes of extinction because of the cataclysmic events, but today's accelerated rate of extinction episodes can be traced only to the influence of human race. Therefore, steps need to be taken to halt such species losses. There is also a need of a well-conceived and dynamic programme of biodiversity estimation, conservation and sustainable utilization.

The over-all estimation of the extent of biodiversity in the form of plant cover and forests in different habitats (including deserts, water bodies, coastal areas, etc.) would come under the purview of Department of Space and Forest Survey of India (MoEF), particularly the former because theirs would be a third party evaluation thus more credible. Periodic (say 5-yearly) reports from these bodies are needed to monitor all habitats for the extent of biodiversity. These data would be useful to take up work on ecorestora-

tion of degraded habitats. Such surveys may also help in calculating the overall quantity of biomass, i.e. all living matter plant, animal and microorganism come under its purview.

The entire *in situ* conservation falls within the mandate of the Ministry of Environment and Forests (MoEF) together with some aspects of *ex situ* conservation, like conservation of complete organisms being attempted in field gene banks in botanic gardens, arboreta, zoos, zoological parks and aquaria (Figure 1). However, use of modern technologies in conservation of organism parts, falls primarily under S&T departments like Department of Agricultural Research and Education (DARE), Indian Council of Forestry Research and Education (ICFRE), and secondarily under Department of Biotechnology (DBT), Department of Scientific and Industrial Research (DSIR) and Department of Science and Technology (DST). These include biological banks for seeds, pollen, sperms, eggs, embryos, tissues, microorganisms and genes (in the form of DNA). A well-managed network of such banks of parts of plants and animals, and microorganisms already exists in the ICAR. This Council has national bureaus on soil and land use, and plant, mammalian, avian, fish and microorganism genetic resources. Some of the bureaus have very large holdings in *ex situ* form. In addition, a large number of collections exist in the Institutes working on crop plants (e.g. wheat, rice, maize, sorghum, potato, tuber crops, sugar cane, cotton, jute, ground nut, gram, soya bean, edible oil crops, pulses, mango,

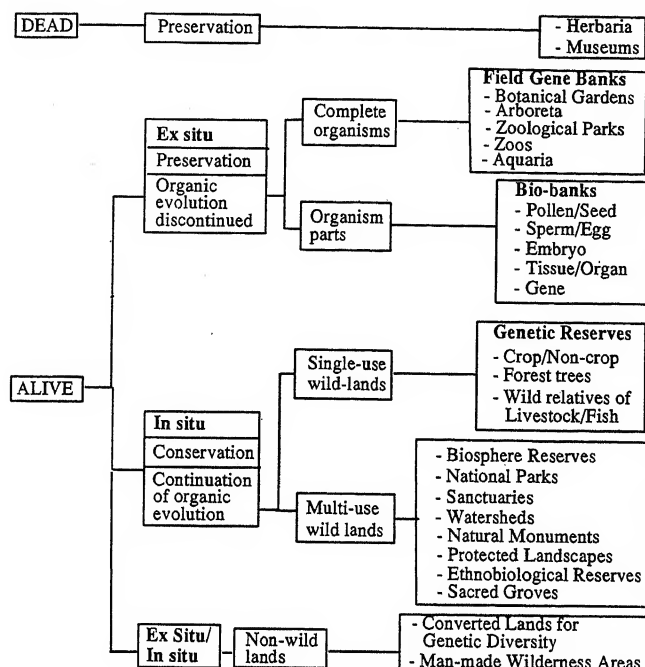


Figure 1. Options of conservation.

citrus, cashew, banana, grape, tobacco, medicinal and aromatic plants, spices, plantation and horticultural crops, mushrooms, orchids and other flowers, etc.). Among animals large collections exist in the case of cattle, buffalo, pig, sheep, goat, poultry, camel, mithun, yak, fish (freshwater and marine), aquaculture, lac, etc. In these Institutes there is also basic S&T infrastructure and capability, together with long-term funding available for this purpose. Additions/replenishments have to be made carefully and should also include all the important endangered and rare species; and ancestral and other related species, land races and primitive cultivars of agricultural crops and domesticated animals for purposes of breeding better types. A network of such banks has to be organized to save the relevant materials under threat of endangerment or extinction. This network should act as Native Germplasm Saver Societies of old and discarded varieties.

Biodiversity in India

The Botanical and Zoological Surveys of the country have estimated that as of today India's biodiversity constitutes 126,188 species¹. These cover all the five kingdoms, namely *Monera*, *Protista*, *Fungi*, *Animalia* and *Plantae*. According to the World Conservation Monitoring

Centre², 1,604,000 species have been described at the global level. Thus India accounts for 8% of the global biodiversity existing in only 2.4% land area of the world.

The country has a coastline of over 7516 km long, a sizeable exclusive economic zone (2.15 million km²) and a large shelf area (0.13 million km²). EEZ is about two-third of the area of main land. Marine areas are by and large still to be systematically charted for biodiversity. There is an abundance of seaweeds, crustaceans, molluscs, corals, fish, reptiles and mammals.

Biodiversity exists at three major levels: *genetic diversity*, *species diversity* and *ecosystem diversity*. *In situ* conservation at the species and ecosystem levels of diversity fall under the purview of MoEF. The first level (genetic diversity) involves the actual utilization and is the concern of DARE, DBT, ICFRE and to a small extent, of DSIR and DST. Thus from genes to ecosystems, there is indeed a continuum³.

Of late, there has been increasing preference and demand for biodegradable products obtained from different forms of biodiversity. Excessive demand for such products would surely lead to bio-depletion of natural biota. There is not only an urgent need for the bioenrichment of the depleted species, but also for evolu-

ing strategies that would prevent bio-impoverishment in natural habitats. MoEF is principally responsible for this and has to harness professional expertise across the whole spectrum for this purpose. Unfortunately, this has not happened so far because these are very few science and technology-based ecorestoration programmes.

Biogeographical provinces

Udvardy⁴ recognized eight realms in the biosphere of the earth. These have been discerned based on a holistic approach. Each realm is infact a complex of related Biogeographical Provinces which number 193. India falls in two realms, with a total of 12 Biogeographical Provinces⁵, which are listed below:

- Palaearctic realm: Tibetan (Ladakh), Himalayan Highlands.
- Indo-Malayan realm: Malabar Rain Forest, Bengalian Rain Forest, Indus-Ganges Monsoon Forest, Assam-Burman Monsoon Forest, Mahanadian, Coromandel, Deccan Thorn Forest, Thar Desert, Laccadive Islands, and Andaman and Nicobar Islands.

The foregoing 12 Biogeographical Provinces have six broad ecosystem types⁵. These are: Tropical Humid Forest, Tropical Dry or Deciduous Forest (including Monsoon Forest or Woodlands), Warm Deserts and Semi-deserts, Cold Winter (Continental) Deserts, Mixed Mountain and Highland System (with complex zonation) and Mixed Island Systems. This classification does not take into account the marine ecosystems and the interface between land and sea, and freshwater and sea. Such interfaces are also rich in biodiversity.

Rodgers and Panwar⁶ have done a detailed exercise, taking into account all the previous classifications including that of Meher-Homji⁷. According to them, the country can be divided into 10 Biogeographical Zones and 25 Biotic Provinces. These are: Trans-Himalayan (Ladakh); Himalayan (North-West, West, Central and East Himalaya); Desert (Kutch and Thar); Semi-desert (Punjab and Gujarat-Rajwara); Western Ghats (Malabar Coast and Western Ghat Mountain); Deccan Peninsula (Deccan Plateau South, Central, Eastern, Chhota-Nagpur and Central Highlands); Gangetic Plains (Upper Gangetic and Lower Gangetic

Plains); North-East India (Brahmaputra valley and Assam hills); Islands (Andaman, Nicobar, Lakshadweep Islands) and Coasts (West Coast and East Coast).

These authors⁶ have taken into account 'land-planning regions of India, largely on geomorphological considerations'. The underlying rationale has been the mega animal part of India's wildlife, rather than the sum total of India's biodiversity. They feel 'some species are characteristic or indicators of certain habitats (e.g. pheasants in temperate Himalayan communities); other species (such as tiger) are dominant member of communities. Ensuring the long-term survival of such animals means that the communities and habitats are also protected'. However, they feel that, for such purposes, 'in general, animals are used more often than plants, mammals are used more than other animal groups, and larger species are used more than smaller ones'.

The report has also brought out that in 1987, there were 54 national parks and 372 sanctuaries with a total area of 109,652 km² or 3.3% of the area of the country. After their review and identification of new sites, they recommended that the country should have 148 national parks and 503 sanctuaries which totals to 151,342 km² or 4.6% of the country's total area.

The report of Rodgers and Panwar⁶ is indeed an excellent effort but takes a traditional view of wildlife. However, today wildlife is regarded as a part of overall biodiversity so as to make the former more holistic. It must encompass the whole gamut of plants, animals and micro-organisms. This change is necessary, because the traditional concept of wildlife is rather restricted in outlook. This laudable report is now a decade old and MoEF has yet to take a decision on the recommendations and publish the report for wider circulation and use.

Hotspots in India

Among the 18 hotspots in the world⁸ two are in India. These are two disjunct areas: Eastern Himalaya and Western Ghats. Their floral wealth is particularly rich so is their endemism not only in flowering plants but also in reptiles, amphibians and swallow-tailed butterflies. Western Ghats have endemic mammals as well.

Apart from the two foregoing mega 'hotspots', 26 endemic centres have been identified by Nayar⁹. These are: Karak-

oram and Ladakh of Kashmir Himalaya; Kumaon-Garhwal Himalaya; Siwaliks; Terai; Sikkim Himalaya; Arunachal Pradesh; Lushai Hills; Tura-Khasi Hills; Aravallis; Chhota-Nagpur Plateau; Panchmarhi-Satpura Range; Simlipal and Jey-pore Hills of Orissa; Bastar and Koraput Hills; Vizagpatnam Hills and Araku Valley; Tirupati-Cuddappa Hills; Marathwada Hills; Saurashtra Kutch; Mahabaleshwar-Khandala Ranges of W. Ghats; Agumbe-Phonda Ranges of W. Ghats; Ratnagiri and Kolaba Ranges; Nilgiris, Silent Valley and Wynad; Anamalais of W. Ghats; Palni-Yercaud; Kalakad and Agasthyamalai Hills of W. Ghats; Andaman Island; and Great Nicobar Island.

The northeastern region is the home of some botanical rarities. One of these is *Sapria himalayana* which is a parasitic angiosperm and has been sighted only twice since 1836. The flowers are about 35 cm across and buds are about the size of a grape fruit. Besides this, Sahni¹⁰ has enumerated several such vanishing taxa. The region is meeting ground of Indo-Malayan and Indo-Chinese biogeographical realms as well as Himalayan and Peninsular Indian elements. It may be recalled that it was here that the Peninsular plate struck against Asian land mass, after it broke off from Gondwanaland. It is, therefore, not surprising that the northeastern India is the region where a large number of primitive angiosperm families are also found. These are: *Magnoliaceae*, *Degeneriaceae*, *Himantandraceae*, *Eupomatiaceae*, *Winteraceae*, *Trochodendraceae*, *Tetracentraceae* and *Lardizalabaleaceae*. The primitive genera are: *Alnus*, *Aspidocarya*, *Betula*, *Decaisnea*, *Euptelea*, *Exbucklandia*, *Haematocarpus*, *Holboellia*, *Houttuynia*, *Magnolia*, *Mangelia*, *Pycnarrhena* and *Tetracentron*¹¹. Takhtajan¹² was led to believe that this region along with contiguous regions is the cradle of flowering plants. Furthermore, Janaki Ammal's¹³ outstanding cytogeographic work has shown that northeast India, together with contiguous region of Chinese provinces of Yunnan and Szechwan, is a very active centre of organic evolution. This has now been confirmed by studies in South East China where an altogether new large mammal (*Muntiacus gongshanensis*) and four new genera (*Xizangia*, *Sinoleontopodium*, *Sindoxa* and *Tetradoxa*) of flowering plants have been discovered¹⁴. Such is the biological riches of NE India and the

adjoining SE China. This region needs special attention.

Although the two areas (North Eastern Himalaya and Western Ghats) are today disjointed having their own characteristic flora and fauna, the following species are common to both^{15,16}. *Ternstroemia japonica*, *Rhododendron arboreum*, *Hypericum hookerianum*, *Thalictrum javanicum*, *Cotoneaster buxifolia*, *Parnassia wightiana*, *Lonicera ligustrina*, *Gaultheria fragrantissima*, *Symplocos lauriana*, Himalayan and Nilgiri Tahr, Nilgiri Pine Marten, laughing thrush (associated with genus *Rubus*), great pied hornbill, frogmouths, fairy blue bird, lizard hawk and rufous bellied hawk-eagle. The probable explanation for the presence of common species between the two disjunct regions is an indication of their being Pleistocene relicts. According to this view, during Pleistocene glaciation, temperate flora and fauna moved south. On retreat of the glaciation, temperate relicts were left at higher altitudes of the southern mountains and continuous distribution between northeast and southwest India was lost after the Pleistocene glaciation. This is the most plausible explanation⁷.

According to Hora¹⁷, there is also resemblance in fish fauna between the two disjunct areas. However, he advanced Satpura Hypothesis, which envisaged movement of Assam flora and fauna through Satpura System to Western Ghats. Whatever be the explanation, the fact remains that the northeast and southwest floras and faunas have some degree of commonality. The common species listed above need detailed genetic study including genetic fingerprinting to establish the relationship between the two groups of disjunct biota in space and time.

In the Indian subcontinent, five sites have been recognized internationally that are not only rich but are also priority sites for data sheet treatment². These are: Agasthyamalai Hills, Nallamalais, Nilgiri Hills, Namdapha, and Nanda Devi. Agasthyamalai and Nilgiri Hills can be categorized as distinct floristic provinces, often covering a very wide area. Together these constitute a centre of plant diversity and/or endemism covering the whole region. For conservation to be effective, a network of smaller reserves needs to be established because it may be impracticable to protect the entire area. Namdapha and Nanda Devi are discrete geographical areas needing conservation.

Endemism and extinction

The most reliable work on endemism in flowering plants of India has been done by Chatterjee¹⁸. Most of the subsequent work has depended on this detailed study. According to him, there are 6850 endemic species in India out of which 3165 (about 50%) occur in the Himalaya. Among the largest genera in India are *Impatiens* and *Primula* with a high degree of endemism. The former has 189 species, about 112 of which grow in the Himalayan belt, while 77 species in Western Ghats, with only one species (*I. balsamina*) common to the two disjunct regions. *Primula* has 162 species out of which 148 are endemic¹⁸. Endemic birds are found in western Himalaya, Indus valley, Western Ghats, eastern Himalaya, Nepal, Bhutan, Bangladesh, Assam Plains and Tirap Frontier with Burma². In higher vertebrates, the country has 12% endemism in mammals, 7% in birds, 40% in reptiles and 53% in amphibia. Andaman and Nicobar has 93% (75 out of 81 species) richness in endemic land snails. Overall, India has 90 species of mammals, 110 species of birds, 158 species of reptiles and 110 species of amphibians endemic in the Indian region^{2,19}.

Data on threatened species may be relatively more reliable. It appears about 1336 species of flowering plants, 39 species of mammals, 72 species of birds, 17 species of reptiles, 3 species of amphibian and 2 species of fish are threatened.

Though no professional studies have been made on the extinction of biota in India, the following species appear to have become extinct in India. These have not been sighted for a long time².

- *Rhodonessa caryophyllacea* (Pink Headed Duck) around 1935 possible cause has been over-hunting.
- *Athene bleinitii* (Forest owlet) around 1914.
- *Ophrysia superciliosa* (Himalayan Mountain Quail) cause was over-hunting. According to Salim Ali it was last sighted in 1876. There is however, a recent unconfirmed report of its sighting in Uttarkhand.
- *Rhinoceros sondaicus* (lesser one-horned rhino) extinct in India but occurs in Java.
- *Acinonyx jubatus venaticus* (Cheetah) extinct in India in 1939, but occurs in Central and Southern Africa and perhaps also in parts of Middle East.

- *Isoetes dixitii* (Isoetaceae) from Maharashtra. Extinct in 1868.
- *Isoetes sampathkumarii* from Karnataka.
- *Lastreopsis wattii* (Aspadiaceae) from Manipur.
- *Ophiorhiza brunonis* (Rubiaceae) from Karnataka and Kerala.
- *Ophiorhiza caudata* from Kerala.
- *Ophiorhiza radicans* from Kerala and Sri Lanka.
- *Wendlandia augustifolia* (Rubiaceae) from Tamil Nadu.
- *Trochetia parviflora* (Sterculiaceae) from Meghalaya.
- *Sterculia khasiana* (Sterculiaceae) from Meghalaya.
- *Eragrostis rottleri* and *E. rangacharli* (Graminae) from Tamil Nadu.
- *Hubbardia hepataneuron* (Graminae) from Karnataka.
- *Dipcadi concanense* and *D. reidii* (Liliaceae).
- *Urginea polyphylla* (Liliaceae).
- *Corypha taliera* (Palmae).
- *Hedychium marginatum* (Zingiberaceae) from Nagaland.
- *Calanthe whiteana* (Orchidaceae) from Sikkim.
- *Prasophyllum colemaniae* (Orchidaceae) from Meghalaya.

Although the loss of foregoing 24 species has come to light, there may be many more species which have become extinct. A systematic study has to be initiated by BSI and ZSI involving the university system.

Centre of origin and diversity

All crop plants and domesticated animals can be traced to their wild ancestors. They have arisen both through inadvertent and deliberate selection by human being. The degree of dependence of these plants and animals on human being is directly proportional to the extent and nature of transformation that has taken place from the wild to the cultivated/domesticated condition. The crop plant genetic resources of the world can be assigned to specific centres of diversity as originally identified by Vavilov²⁰.

Vavilov identified these on the basis of varietal diversity, homologous variation, endemism, dominant allele frequencies and disease resistance. The centres are located in different continents. These have also been referred to as Germplasm Treasures (for details see Khoshoo⁵).

While Vavilov's²⁰ basic conclusions

have stood the test of time, there have been small differences about the number and location of centres of diversity. There is today a general unanimity about 12 centres of diversity²¹. India is one very important centre having contributed to world agriculture at least 167 plant species^{5,21}. Within the overall mega Indian Centre of Diversity as recognized by Vavilov, there are at least nine subcentres of diversity, where wild relatives of cultivated plants still occur²².

One indeed marvels at the intuitive power of our very remote ancestors to have picked up these plants from the wild, and selected these unconsciously and consciously so as to make them far different and highly productive compared to the ancestral stock. Some of these are being cultivated world wide, e.g. rice, sugarcane, cucumber, egg plant, banana, citrus, ginger, etc. Among animals, the three important animals (chicken, cattle and pig) supporting world animal husbandry, chicken (*Gallus gallus*: jungle fowl) is India's contribution. The cultigens and domesticated types both in plants and animals have been crafted meticulously and are different from the ancestral species. Like cultural and developmental diversity, agri-biodiversity is also a part of the creative diversity of human being. All these diversities are mutually supportive and reinforcing.

There are secondary centres of genetic diversity which are environmentally different from the primary centres, where the crops were developed further by human ingenuity. Thus India is a secondary centre of diversification for several species which are very old introductions (may be even pre-Columbian) into the country. Such crops are grain amaranths, maize, red pepper, soybean, potato, oil palm, etc. Similarly, the Indian breeds of exotic and domestic animals like horse, pony, sheep, goat, cattle, etc. are always in demand particularly for their disease resistance and hardy traits. The reason is that these animals are the result of hardiness, adaptation to heat, parasitic stresses, and availability of roughage with low nutritive value and, therefore, these are in demand for breeding purposes in Australia, USA and Latin America.

National Biodiversity Conservation Board (NBCB)

By their very nature, most Indians are

peace-loving and vegetarian, and believe in non-violence which is enshrined deep into their psyche. Thus conservation is basically a part of Indian ethic. It is, therefore, not unexpected that in the historical past, India had a tradition of giving highest attention to wildlife. In the recent times, the country had a powerful Indian Board for Wildlife (IBWL) headed by the then Prime Minister of India, Indira Gandhi. This continued up to her assassination. After her, the Board has met *only once*. What it means is abundantly clear!

There is, however, no doubt that the concept behind IBWL has outlived its utility because there is a need to widen its scope and make it holistic. Even the World Wildlife Fund, from which many rich Indians have been drawing inspiration, was compelled to change its name to World Wide Fund for Nature by sheer circumstances of new and vastly extended knowledge on biodiversity. However, for the sake of continuity and convenience, they continued to have the same acronym: WWF. Theoretically, wildlife means all undomesticated plants and animals, but in practice it was restricted to large mammals, big cats in particular. This was so on account of a feeling that these animals were top of the food chain. The underlying rationale was: if big animals are conserved, *ipso facto* others below them are also conserved. This may not be correct under all circumstances and can, therefore, be a fallacious argument. In India due to the outstanding work of the late Salim Ali, birds have been studied very intimately. There has also been outstanding work done on algae, fungi, liverworts, mosses, ferns, gymnosperms besides angiosperms. The same is true of different groups under the animal kingdom. All this work is scattered and needs to be organized into a modern database.

Today, the concept of wildlife has widened considerably. It includes all biodiversity as a dynamic and interacting system, of which even the local human beings are also an integral part. It is not merely the number of microorganism, plants and animals, but the most important point is the interconnectedness, interrelatedness and interdependence of plants, animals (human beings included) and microorganisms existing as a system. There is also a very definite connection between biodiversity and cultural diversity, together with social, economic, historical, religious and philosophical dimensions.

Such a relationship is mutually reinforcing (Khoshoo, unpublished). Biodiversity is now looked upon as a major renewable resource and hence an important Earth Capital. If we take away biodiversity from the Earth, human beings cannot exist. Conversely, if we were to take away human beings from the surface of earth, biodiversity (except agri-biodiversity which has been created and crafted by human beings) will continue to exist, may be even flourish. Human race must realize this.

This country has done nothing worthwhile, although we made tall claims after signing the Biodiversity Convention at Rio in 1992 and thereafter. It may also be pointed out that biodiversity is not to be mistaken for mere animal welfare and such other populist measures, but there is whole range or scientific, technological, social, economic, ethical, moral and political disciplines involved in it.

Furthermore, throughout rural India, there are innumerable microenterprises at the village level using local biodiversity. The products from the same find their way to cities and even in international market. These are exotic and often exquisite items and are in demand. There have been very few dependable studies on such enterprises. There is need to document and analyse these with a 'cradle to grave' approach. With a little innovation, such studies can be linked not only provincially, but also nationally and some even globally. Sufficient attention has not been paid to these aspects except for the outstanding studies of K. S. Bawa and his colleagues. Their work has attracted world attention. As a whole this area is indeed uncharted and if work is done professionally, it would also give us an inkling about the economic value of the products obtained from our biodiversity. Regrettably, Indian biologists have not played their part well. The vacuum thus caused has been filled by a host of non-biologists. The professional biologist of the country must now take a lead lest the whole area of biodiversity should fall in wrong hands.

Another dimension is that there is considerable illicit trade in wild animal parts and plants, e.g. tiger bones, claws, skin of tiger and other cats, rhino horns, butterflies, orchids, herbal drugs and aromatic plants, sandalwood, etc. It is clear that animal and/or plant producing countries are in developing world in South America,

Meso-America, Africa and South East Asia, but ultimate destination of the traffic in biodiversity is USA, Canada, West Europe, Japan, Middle East and China²³. On paper, India is neither a producing nor a receiving country, but considerable illicit trading in biodiversity comes to light once in a while.

Techniques like DNA fingerprinting can be used to identify such threatened and rare species. This technology has become very important because it enables unequivocal identification of the concerned species. Not only do target species need fingerprinting but also their adulterants. In addition, the diversity within the species needs to be charted and related to their chemical profile particularly in the identification of high-yielding strains of the concerned phytochemicals from drug and aromatic plants. Work on genetic fingerprinting has to be intensified and fingerprints obtained on most cultivated/domesticated and wild biota (human diversity included). All this information has to be collected in the form of a proper database and related to the uses of different cultivars, ecotypes, chemotypes, etc. Such information together with species-specific and even gene-metabolite linked probes will also be of help in standardization of bio-pharmaceuticals and biodiversity-linked intellectual property rights, etc. For some critical species (endangered species like lion and tiger), such a technique will also give us an idea of the extent and nature of genetic variation so as to decide an effective strategy for its conservation based on principles of population genetics and breeding biology.

These sophisticated techniques need to be applied on a systematic basis to genetically, economically and trade-related biodiversity. Such a sophisticated database is going to be also critical to biodiversity conservation and utilization. Besides, it will have tremendous social, economic, ethical, and legal implications.

The foregoing aspects of biodiversity have to be accompanied by teaching and training in Conservation Biology: a new multidisciplinary subject which is becoming increasingly critical to the conservation and sustainable utilization of biodiversity particularly in developing countries. Advanced teaching and training in this discipline has to be started in some chosen conventional and agricultural universities and Wildlife Institutes so as

to generate a cadre of well-trained and knowledgeable conservation biologists.

An important and an integral component of Conservation Biology will be a proper economic valuation of India's biodiversity. In fact it is a part of larger problem of proper economic evaluation of different forms of the Earth's capital (air, water, soil, minerals, etc.). Biodiversity has an indirect and a direct value. Indirect value pertains to the overall biological productivity of an ecosystem, fresh air and water, soil, regulation of climate, and ecotourism for sheer greenery and fresh air and clean water and good viewing of wild animals and plants in their natural habitats. The direct value pertains to the level of community use of timber and whole range of non-wood forest products, genetic resources of crops and domestic animals and their ancestral and other related species. Putting a price tag on our biodiversity will make its loss more understandable because it would be in fiscal terms. At present economic value of wild biodiversity (be it a medicinal or aromatic or fruit plant or whatever) is the cost of travel to collect the same, and no more. Those who make products, make the real money. However, it is the end-user and nature at large who pay the real cost.

Policy decisions regarding biodiversity have to be taken realistically, based on

actual facts. Therefore, there is need to consolidate information and put it in the form of a major database commensurate with the biodiversity wealth of India.

Being predominantly a biomass-based country with largely bioindustrial pattern of development, India's stakes in biodiversity are indeed very high. Our performance in this area has been far from ideal. This is true even in its legal and political dimensions. At any rate, it is not commensurate with the extent and nature of bio-wealth that India owns. There are reasons for this. The result has been that biodiversity which is India's strength has been progressively going by default even when internationally biodiversity has assumed considerable importance. It is high time that Government and scientific and economic communities and responsible social scientists think about it very seriously. The least that can be done is to organize a competent and responsible organization so as to give proper importance and treatment to this wealth for the good of the country.

The information on biodiversity is very dispersed and needs to be consolidated so that the country can reap rich harvests from this important wealth. There is, therefore, a very urgent need of having a comprehensive and a professional National Biodiversity Conservation Board

(NBCB) which can look at various aspects of biodiversity, ranging from environmental, to biological (including agricultural), social, economic, ethical and other related dimensions. The three broad functions (Figure 2) are the establishment of database(s), and management and utilization of India's biodiversity. The National Database(s) will store data on all the five kingdoms. Other equally important databases would deal with agricultural and industrial biodiversity. Here information of wild and human-created and crafted useful microorganisms, domesticated animals and cultivated plants together with their ancestors and related species and other relevant dimensions will be stored. It is indeed gratifying to note that this country already has a chain of very prestigious national bureaus of plant and animal genetic resources under the aegis of ICAR.

Indian Council of Forestry Research and Education (ICFRE) must take up the responsibility of conservation of forest tree germplasm, both under *in situ* and *ex situ* conditions in a meaningful manner^{15,24}. Forest tree germplasm and lack of proper forest tree genetics and breeding programmes have been major lacunae in forestry research and development. This is why our wood productivity is indeed dismal, being the lowest in the world²⁴.

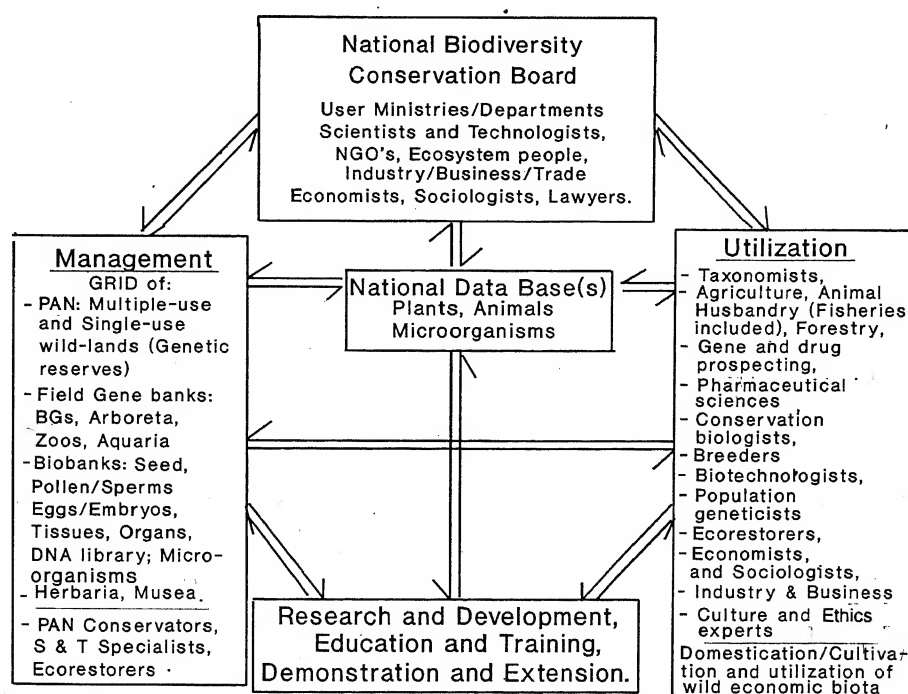


Figure 2. Broad functions of the National Biodiversity Conservation Board.

It may also be pointed out that the Ministries/Departments/Organizations involved with NBCB would be Environment and Forestry, Agriculture, Industries, Commerce and their relevant Departments (DARE, DBT, DSIR, DST), ICFRE and those dealing with commodities like tea, coffee, jute, cotton, silk, tasar, rubber, cashew, coconut, arecanut, phytochemicals, biopharmaceuticals, biocosmetics, bioinsecticides, biopesticides, biofertilizer, etc. The total membership of the NBCB should under no circumstances exceed nine otherwise it will be only a talking body.

Of late, there has been a realization in the industrial countries that local technical knowledge of the indigenous people in the developing countries is not mere collection of myths and voodooos, but distilled knowledge accumulated over millennia. Such knowledge is based on large scale trial and error and intensive observation. Regrettably, this knowledge is regarded as 'ownerless' and is taken for granted as *Common Heritage*. There is now major effort by industrial countries to own all such 'ownerless' knowledge and resources. After its updating and refining, in industrial countries through the application of relevant upstream S&T, this knowledge would be regarded as *innovation* and *intellectual attainment* and would become patentable. It would then be rewarded and awarded as private property and become available to only those who can pay the high price. All this has led to renewed emphasis on ethnobiology, ethno-medicine and ethno-pharmacology. This aspect of biodiversity has also opened up new vistas in ecology, biology, economics, micro-enterprises, anthropology, linguistics (particularly local languages), community development, conservation, etc.

In view of the foregoing, *the time has come that the country must declare biological diversity a National resource, its conservation and sustainable utilization a National goal and National priority*. The functions of NBCB have been worked out¹⁵ and need to be refined further. These need to be periodically looked at indepth and updated. An illustrative list of functions is given below:

- Formulate a National Policy on conservation and utilization of India's biodiversity and agrobiodiversity together with a time-bound plan of action.
- Inventorize India's biodiversity.

- Establish minimal database(s).
- Review existing PAN (Protected Areas Network), identify gaps and draw criteria for identification of new protected areas.
- Examine tenurial security of PAN to ensure conservation in perpetuity.
- Prepare plans for management of PAN, ensuring stake and involvement of people.
- Support PAN with adequate number of genetic reserves, botanical and zoological gardens, arboreta and aquaria, and biological banks of organism parts including DNA.
- Draw plans for ecorestoration of degraded habitats.
- Draw plans for *ex situ* conservation together with rehabilitation of endangered species (e.g. tiger, rhino, lion, pheasants, butterflies, wild medicinal and other economic plants, etc.) based on genetic-evolutionary considerations.
- Draw criteria for endangerment of species leading to extinction together with causes for the same and suggest remedial measures.
- Draw conservation and sustainable utilization plans regarding hitherto neglected areas like marine biodiversity, forest tree genetic resources and microorganisms.
- Following principles of population and evolutionary biology and genetics and breeding, domesticate wherever necessary, wild biota that are in demand in trade, e.g. medicinal and aromatic plants, ornamentals, butterflies, fish, fur animals, botanical and zoological rarities and teaching materials.
- Draw plans for meaningful involvement of local people in conservation effort and in community biodiversity programmes.
- Establish centres for research and development, teaching and training and demonstration and extension in conservation biology, ecorestoration of habitats; economic value of ecosystems, species and genes; trade in biodiversity, particularly in endangered species; microenterprises at the village level; and indigenous people and their local technical knowledge.
- Build a cadre of PAN conservators and S&T specialists.
- Establish centre(s) of study for legal and policy aspects relating to conservation and utilization of biodiversity.
- Guarantee financial support.

Finally, it may pointed out that in its wider context, the poor and struggling developing countries of the world in tropical/subtropical belt are particularly rich in biodiversity, but are very poor in its utilization using modern science and technology. There are definite reasons for this²⁵. There is also an immediate need for an indepth discussion for forging ahead an alliance between all or most biodiversity-rich but technology-poor developing countries so as to deal with biodiversity-poor but technology very rich industrial countries in an effective and a gainful manner²⁶.

If this is not done, the onslaught of concealed compulsions from industrial countries will keep developing countries (rich in biodiversity and local technical knowledge) in *permanent bondage*. Therefore, the most urgent need is to professionalize and technicalize the whole area of biodiversity, if not the whole area of environment.

Indian Bioresources Council

Over the years there have been suggestions made from India and abroad regarding organizing inter-ministerial and an all-encompassing Indian Bioresources Council (IBC). Bioresources as a whole are indeed very critical for the development of India, because, as pointed out earlier, this country is essentially a biomass-based and predominantly rural country, where the pattern of development has to be bio-industrial rather than purely industrial. There are already several Councils under Government of India dealing with bioresources (including human being), e.g. Indian Council of Agricultural Research, Council of Scientific and Industrial Research, Indian Council of Medical Research, Indian Council of Social Sciences Research, etc. In addition, there are Councils on Ayurveda, Unani, Siddha systems of medicine. The basic raw material for all these councils is biodiversity of sorts (including human). These councils have rendered yeoman service. Having IBC over these, would, therefore, be adding a layer of administrative hassles. It would be counter-productive. However, a NBCB will be more pointed and focused organization to oversee in entirety conservation and utilization of the principal bioresource, i.e. biodiversity.

Note added in proof: An important publication on Hotspots of Endemic Plants by M. P. Nayar has been just published by the Tropical Botanic Garden and Research Institute. This is an update on Chatterjee's (1939) paper referred to in the text.

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Indian cycads cry for protection

E. J. H. Corner who was professor of Tropical Botany in the Botany School, University of Cambridge has aptly said, 'But the forests which show how trees were made are dying, they are vanishing nowhere faster than from the alluvial plains where the vestiges of the last creative phase of plant life that prepared the way for the modern world may survive.... Before machines the forest is defenceless. Human progress is clearing it with gathering speed to plant crops for quick returns'. It is, therefore, necessary to conserve the plant cover of tropical forests, and therein specially the cycads, need to be conserved. Being relics of a bygone age they are handicapped by their slow growth, relatively short stature, disadvantageous dioecism, sex ratio problems including less frequent coning of female individuals, frequently non-synchronous production of male and female cones, their inefficient pollination mechanisms, shedding of seeds with immature embryos and a long period of about 10 years required by seedlings to attain puberty for producing reproductive parts. Accordingly in various native coun-

tries of cycads, except India, there is public and governmental awareness about the need of conserving cycads against human interference.

My visits to Beaulieu-sur-Mer in southern France in 1987, to Australia in 1990 and my recent visit to China in May 1996 to attend the International Conferences on Cycad Biology have convinced me that other countries are far ahead of India in the conservation of cycads. The Cycad Societies of USA, South Africa and China and the Palm and Cycad Societies of Australia and New Zealand have played a vital role in creating such awareness about protecting their cycads *in situ* (in the areas of their occurrence) and *ex situ* (under cultivation in gardens, parks and in private collections).

In situ conservation

As a result of this awareness all native countries have started protecting their natural populations of diverse cycads in protected areas. In China alone there are about 20 or more natural population reserves where cutting of any parts of cycads or even the collection of fallen

seeds is prohibited. Australia and Central America have also taken steps to protect their rare cycads. South Africa in particular has already enacted legislation to protect its cycad populations and to punish the guilty with fine and imprisonment. India, on the other hand, cannot claim even a single Cycad Reserve anywhere in the country, perhaps, because we are over-emphasizing biotechnology, agricultural production and applied sciences.

Ex situ conservation

Despite the limitations of cultivated collections of cycads, lasting conservation is offered to cycads, by various botanical gardens of the world. Some of these are situated in temperate or cold temperate countries (where the climate is not favourable for growth of cycads in the open), e.g. in the Royal Botanic Gardens at Kew in UK, the St. Petersburg (Leningrad) Botanical Garden of Russia and the Glasnevin Botanical Garden of Ireland and they all have stupendous collections of the cycads of the world. Many South African Botanical Gardens,



Cycas circinalis plants in the Nagamangala area. The top of one of the trees has been cut out.

the New York Botanical Garden and the Fairchild Tropical Garden of Miami, Florida need special mention. Practically every important city in Australia has a botanical garden and many of them give particular attention to cycads, specially the Royal Botanic Gardens in Sydney. Many of the above mentioned gardens are also involved in cycad research. The Chinese too seem to be ahead of us in the *ex situ* conservation of their cycads. Their South China Botanical Garden, Panzhihua Institute of Horticulture, Panzhihua Park and Shenzhen Fairy Lake Botanical Garden have special collections of cycads. In particular, the Shenzhen Fairy Lake Botanical Garden has an international centre for *ex situ* protection of cycads where not only Chinese cycads but cycads from other countries have been introduced. Whereas we cannot claim even a single botanical garden, with a good collection of our own cycads, or of diverse cycads of the world.

Indian cycads and the need of their conservation

Indian region can claim only six species

of the genus *Cycas* as its natives; *Cycas beddomei*, *C. circinalis*, *C. nathorstii*, *C. pectinata*, *C. rumphii* and *C. siamensis*. Out of these, *C. beddomei* is one of the most circumscribed endemics among all cycads growing only in the Cuddapah Hills of Andhra Pradesh. It is accordingly listed in Appendix I of the Convention on International Trade in Endangered Species of wild fauna and flora (CITES) which is administered by the United Nations Environment Programme. Its inclusion in Appendix I indicates that it is one of the endangered plants of the world and international trade in these plants or their parts is prohibited by CITES. However, their protection in India is our responsibility.

Our second species, *C. circinalis*, is relatively widely distributed from Kerala northwards up to Orissa. However, it has at least three varieties having a more restricted distribution: *C. circinalis* var. *circinalis* grows in the Malabar region, var. *swamyii* grows in the Hassan District of Karnataka and var. *orixensis*, in the Mals of Puri.

Among the remaining species, *C.*

nathorstii is endemic to Sri Lanka, *C. pectinata* occurs in the Someshwar Hills of Bihar, the Assam region, Eastern Nepal and Sikkim Terai, *C. rumphii* grows in the Andaman and Nicobar Islands and Sri Lanka and *C. siamensis* is reported rarely from Manipur and Bhutan.

The above-mentioned restricted populations of our cycads are regularly ravaged by inhabitants of the surrounding areas for use of their leaves, the entire crowns of leaves and apical parts of stems for decorations of their temples, churches or graves. The plants are also used for medicine and food. Thatching and preparation of mats and broom sticks are other uses of cycad leaves. Cones are also used for driving away bed bugs or rice bugs.

However, the worst calamity which is overtaking our cycads is the wanton destruction of their entire populations for acquiring their habitats for human habitations including dwellings, fields and roads or for quarrying stones. In this process the habitats of cycads are rapidly shrinking and the species are threatened with extinction as happened in the case of *Encephalartos woodii* in Africa where only male plants survive in cultivation. These are vegetatively propagated from a multistemmed male which was the only plant ever collected from nature in 1895. *C. beddomei*, despite being listed in Appendix I of CITES as one of the most endangered cycads, with an embargo on its international trade, has been mercilessly destroyed in Tirupathi where an entire hillside inhabited by its beautiful plants was cleared of the vegetation by the authorities of the Tirupathi Devasthanam for their buildings, roads and gardens.

At this point I can also recall the merciless cutting of a number of large female trees of *Cycas revoluta* growing in the old Government House of Allahabad. Each of these had about 60 or more large and small branches all round and these grew from the ground surface to a height of about four metres. The thickest of their trunks must have been about a metre in diameter. Their sight was so amazing that I used to take my students and botanist friends to see them. Suddenly one day I came to know that all of them had been cut down and the branches thrown away because the State Government had decided to convert the Allahabad Government House into the Moti Lal Nehru Medical College. The trees must have been planted at least

about two hundred years earlier. Obviously, no one, not even the Government cares for such valuable monuments of nature. If the authorities concerned had cared the trees could have been carefully dug out with the roots and transplanted. In other countries old cycads are valued and protected wherever they grow. The Chinese and Japanese plant cycads in their temples and some of their famous oldest and largest trees are situated there, e.g. the Ryugeji Temple near Tokyo has many old trees of *Cycas revoluta*. Surely we could emulate this practice in our temples and churches instead of decorating them with cut leaves and crowns.

Suggested methods for conservation of Indian cycads

The manner in which India could embark on a programme for the protection of its cycads is envisaged as follows:

1. A survey of the natural areas and

population counts of all our cycads in an All-India basis along with a search for new forms.

2. Protection of their habitats by declaring some of their habitat areas as 'Cycad Reserves'.

3. A programme should be embarked for the education of the public in the areas of our cycads and also elsewhere on the need of protecting our cycads wherever they grow, and, if necessary, we should embark on legislation for the protection of cycads in nature and old plants in cultivation. We should have a Cycad Society, if necessary subsidized by the Government, for education and research on cycads like the cycad societies of other countries. This could help us in developing our much needed research on different aspects of cycads.

4. India should have a few cycad gardens and special cycad sections in all botanical gardens. Indeed India needs many more botanical gardens in different parts of the country to preserve the diversity of its flora.

We could encourage cultivation of cycads in parks, road sides and private gardens by providing a subsidy for the purpose.

In this connection it is important to point out that Indian scientists sitting in positions of power in Government Departments should take effective measures for the protection of our endangered species like *Cycas beddomei* and not merely write articles about their imminent extinction. Such statements should also be backed by actual surveys of their distribution and numbers and by the establishment of 'plant sanctuaries'. India has a number of 'National Parks' for the protection of animals facing extinction but not a single one for the protection of its endangered plants. Perhaps, I could also blame myself for this situation since I repeatedly refused positions in Government Departments.

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SCIENTIFIC CORRESPONDENCE

Evolution of the nasuta-albomicans complex of *Drosophila*

During the last two decades, the *nasuta* subgroup of the *immigrans* group of *Drosophila* has attracted the attention of taxonomists, cytogeneticists, biochemists, molecular biologists and evolutionary biologists. The *nasuta* subgroup has evolutionary peculiarities, which include little morphological differentiation despite their distribution over an enormous territory, the ability to intercross in the laboratory, often producing fertile offspring and substantial chromosomal evolution¹. The story started with the report of *D. nasuta* by Lamb² from Seychelles islands. Subsequently, morphologically almost similar forms found in different parts of southeast Asia were studied by Wilson *et al.*³. Further investigations by Indian⁴⁻⁷ and Japanese researchers^{8,9} resulted in establishing the 14 different species of the *nasuta* subgroup. The females of all the species are morphologically indistinguishable, while three types of males are recognized^{3,8}. On the basis of the morphology of the frons and orbits of the males, three morphophenotypic complexes have been identified^{3-5,8}. The males of

D. nasuta, *D. albomicans*, *D. kepulauan*, *D. kohkoa* and *D. neveifrons* with complete silvery frons constitute 'frontal sheen complex'. On the other hand, the silvery sheen is restricted to the sides of the frontal orbits in the males of *D. s. sulfurigaster*, *D. s. bilimbata*, *D. s. albos-trigata*, *D. s. neonasuta* and *D. pulaua*, constituting the 'orbital sheen complex'. The third complex shows absence of pollinosity of frontal region and includes *D. pallidifrons*, taxons F, J, and I.

In the frontal sheen complex, *D. nasuta* and *D. albomicans* are a pair of chromosomal allopatric races (earlier they were treated as biologically valid reproductively isolated species, and hence were given different names) with $2n=8$ and $2n=6$, respectively¹⁰. The difference in the diploid number is because the sex chromosomes and the 3rd autosome exist as independent acrocentric entities in *D. nasuta*, whereas in *D. albomicans*, these two components of the karyotype exist as one unit in the form of a metacentric chromosome. In spite of the difference in diploid number, there is similarity

between the corresponding chromosomes of *D. nasuta* and *D. albomicans*, as seen in the polytene chromosomes of F_1 hybrid larvae¹¹. They also show variation not only in chromosome number, but also in the quantum of heterochromatin present in different chromosomes¹², and in the organization of micro (dot) chromosomes¹³. Because of this difference, the size of the respective chromosomes of *D. nasuta* and *D. albomicans* differ, and this helps to distinguish the chromosomes of these races in their hybrids. In spite of such karyotypic differences, the F_1 hybrids with $2n=7$, (4 chromosomes of *nasuta* and 3 of *albomicans*) are fertile and the hybrid progeny can therefore be maintained for generations. The ability to identify the parentage of each chromosome in the hybrid karyotypes and to indefinitely maintain the hybrid populations have formed the basis for long range evolutionary studies of the present authors^{11,14}.

Hybrids of *D. nasuta* and *D. albomicans* have been maintained in our laboratory for over a decade. The F_1 with $2n=7$

breeds to yield F_2 hybrid population, which is karyotypically heterogeneous. In addition to individuals with *nasuta*-like, *albomicans*-like, and F_1 -like karyotypes, other combinations are also seen¹⁵. Such karyotypic mosaicism persists for many hybrid generations, while in some hybrid lineages, karyotypic variability disappears and the population has one type of stabilized karyotype. During the evolution of a stabilized karyotype, some of the parental chromosomes are eliminated, while others are retained. The time taken for a karyotype to stabilize ranges from 20 to over 150 generations and, in a few cases, heterogeneity is maintained without attaining karyotype stability (unpublished). Stabilized hybrid karyotypes are invariably a combination of chromosomes inherited from both the parents. Thus, a new karyotype, called cytorace was developed in our laboratory having chromosomes of both *nasuta* and *albomicans*. There are four such cytoraces^{16,17}. The males and females of these four cytoraces along with *D. nasuta* and *D. albomicans* were intercrossed and they yielded 30 new hybrid lines, which are being maintained (unpublished). Periodically, the karyotypes of these hybrid populations passing through different hybrid generations are being analysed along with other features such as mating preference¹⁸, population fitness^{19,20}, ecogenetic difference²¹, if any, among different hybrid lineages.

At present, as a result of interracial hybridization experiments for over a decade, we have created 16 different races derived from intermixing the genomes of *D. nasuta* and *D. albomicans* (Table 1) (unpublished). Details of the role of different chromosomes in the evolution of these races will be discussed elsewhere. In brief, all these hybrid populations have chromosomes derived from *nasuta* and *albomicans*. Therefore, cytologically these races are closely related to one another. They differ from one another in the hybridization path through which they have evolved and are evolving. These genetically diverged cytologically parsimonious races, evolved under laboratory conditions, constitute a new assemblage within the 'frontal sheen complex' of *Drosophila*, called the *nasuta*-*albomicans* complex. This new complex stands parallel to the three naturally evolved morphophenotypic complexes of the *nasuta* subgroup mentioned earlier.

Chang *et al.*¹ analysed mitochondrial DNA of the members of the *nasuta* subgroup and suggested that most of the lineages of this subgroup diverged from each other between 1 and 2 Myr. On the contrary, the newly established *nasuta*-*albomicans* complex has evolved/diversified within a span of one decade in the laboratory. The members of the three naturally evolved complexes of the *nasuta* subgroup exhibit genetic divergence without morphological differentiation. In this regard, the laboratory evolved *nasuta*-*albomicans* assemblage is comparable to

these natural groups. The emergence of this new assemblage is through interracial hybridization between *D. nasuta* and *D. albomicans*, followed by hybrid recombination and karyotype stabilization. The event of hybridization has hastened the process of the formation of new differentiating populations and it justifies the opinion of Dobzhansky *et al.*²², that hybridization is an evolutionary catalyst. It might have taken millions of years in nature to evolve a group of such differentiating races, but it has taken just a decade in the present experimental setup.

Table 1. Summary of the karyotypes of cytoraces along with their parental races under laboratory conditions. The superscripts 'n' and 'a' indicate *nasuta* and *albomicans* respectively

Name	Karyotypes
<i>D. nasuta</i>	(♂ & ♀) - $2n = 8 - X^n X^n Y^n 2^n 2^n 3^n 3^n 4^n 4^n$
<i>D. albomicans</i>	(♂ & ♀) - $2n = 6 - X3^a X3^a Y3^a 2^a 2^a 4^a 4^a$
Cytorace 1	(♂ - $2n = 7 - 2^n 2^a X3^a Y^n 3^n 4^n 4^n$; and ♀ - $2n = 6 - 2^n 2^a X3^a X3^a 4^n 4^n$)
Cytorace 2	(♂ - $2n = 6 - 2^n 2^a X3^a Y3^a 4^n 4^n$; and ♀ - $2n = 6 - 2^n 2^a X3^a X3^a 4^n 4^n$)
Cytorace 3	(♂ - $2n = 8 - 2^n 2^a X^n Y^n 3^n 3^n 4^n 4^n$; and ♀ - $2n = 8 - 2^n 2^a X^n X^n 3^n 3^n 4^n 4^n$)
Cytorace 4	(♂ - $2n = 7 - 2^n 2^a Y3^a X^n 3^n 4^n 4^n$; and ♀ - $2n = 8 - 2^n 2^a X^n X^n 3^n 3^n 4^n 4^n$)
Cytorace 5	(♂ - $2n = 7 - 2^n 2^a X3^a Y^n 3^n 4^n 4^n$; and ♀ - $2n = 6 - 2^n 2^a X3^a X3^a 4^n 4^n$)
Cytorace 6	(♂ - $2n = 7 - 2^n 2^a Y3^a X^n 3^n 4^n 4^n$; and ♀ - $2n = 8 - 2^n 2^a X^n X^n 3^n 3^n 4^n 4^n$)
Cytorace 7	(♂ - $2n = 7 - 2^n 2^a X3^a Y^n 3^n 4^n 4^n$; and ♀ - $2n = 6 - 2^n 2^a X3^a X3^a 4^n 4^n$)
Cytorace 8	(♂ - $2n = 7 - 2^n 2^a X3^a Y^n 3^n 4^n 4^n$; and ♀ - $2n = 6 - 2^n 2^a X3^a X3^a 4^n 4^n$)
Cytorace 9	(♂ - $2n = 6 - 2^n 2^a X3^a Y3^a 4^n 4^n$; and ♀ - $2n = 6 - 2^n 2^a X3^a X3^a 4^n 4^n$)
Cytorace 10	(♂ - $2n = 8 - 2^n 2^a X^n Y^n 3^n 3^n 4^n 4^n$; and ♀ - $2n = 8 - 2^n 2^a X^n X^n 3^n 3^n 4^n 4^n$)
Cytorace 11	(♂ - $2n = 6 - 2^n 2^a X3^a Y3^a 4^n 4^n$; and ♀ - $2n = 6 - 2^n 2^a X3^a X3^a 4^n 4^n$)
Cytorace 12	(♂ - $2n = 6 - 2^n 2^a X3^a Y3^a 4^n 4^n$; and ♀ - $2n = 6 - 2^n 2^a X3^a X3^a 4^n 4^n$)
Cytorace 13	(♂ - $2n = 6 - 2^n 2^a X3^a Y3^a 4^n 4^n$; and ♀ - $2n = 6 - 2^n 2^a X3^a X3^a 4^n 4^n$)
Cytorace 14	(♂ - $2n = 7 - 2^n 2^a Y3^a X^n 3^n 4^n 4^n$; and ♀ - $2n = 8 - 2^n 2^a X^n X^n 3^n 3^n 4^n 4^n$)
Cytorace 15	(♂ - $2n = 8 - 2^n 2^a X^n Y^n 3^n 3^n 4^n 4^n$; and ♀ - $2n = 8 - 2^n 2^a X^n X^n 3^n 3^n 4^n 4^n$)
Cytorace 16	(♂ - $2n = 8 - 2^n 2^a X^n Y^n 3^n 3^n 4^n 4^n$; and ♀ - $2n = 8 - 2^n 2^a X^n X^n 3^n 3^n 4^n 4^n$)

Races with $2n = 7$ in males show a few sterile aneuploids.

Even though some of the races appear to have similar chromosomal complement, the hybridization path and the phylogenetic history of chromosomes differ.

The present investigations can be considered as an evolutionary experimentation through hybridization under laboratory conditions. These races which are at different stages of evolutionary divergence offer a rare opportunity to study the process of raiation (speciation ?), under laboratory conditions. The *nasuta-albomicans* complex of *Drosophila*, the members of which are in the process of evolving, is a unique model system to witness the process and the pattern of sibling raiation/speciation as well as the analysis of chromosomal and molecular basis of raiation.

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Evidence for positive assortative mating within *Drosophila bipectinata*

Sexual (or ethological) isolation, which is a premating barrier to gene exchange between Mendelian populations, is the most important class among the different ways of reproductive isolation in animal species. This plays an important role in evolution. The phenomenon of sexual isolation has been extensively studied in the genus *Drosophila* and has been found to be widespread^{1,2}. Incipient reproductive isolation occurring between geographic strains of the same species has been reported in many *Drosophila* species, which corroborates the hypothesis that incipient isolation originates as a side-effect of genetic divergence¹⁻³. Although a lack of assortative mating between mutant and wild type *D. melanogaster* has been reported⁴⁻⁶, selective mating has also been found in some cases^{7,8}. Rendel⁹ found selective mating (non-random) between yellow mutant and wild type with yellow males.

Drosophila bipectinata is a member of the *bipectinata* species complex of the

ananassae subgroup of the *melanogaster* species group. Population and behaviour genetical studies in this species have been initiated in our laboratory¹⁰⁻¹⁴. *D. bipectinata* shows incomplete sexual isolation with other members of the *bipectinata* complex¹⁵. It is also characterized by incipient sexual isolation between different geographic strains¹¹. Spontaneous mutations have been detected in this species¹⁶⁻¹⁸. Effects of mutations on mating propensity and pattern of mating have also been tested¹⁹⁻²¹. We detected flies with bilateral outgrowths on thorax which is a unique phenotypic change in *D. bipectinata*¹⁷. A separate stock of mutant with outgrowths could be established. During the present study, we tested the pattern of mating between wild type and mutant (possessing bilateral thoracic outgrowths) *D. bipectinata* and the results are reported here.

During the present study, the wild type (TD) and mutant (OG) stocks of *D. bipectinata* were used. In mutant stock, flies

possess bilateral outgrowths on thorax. Originally, this unique phenotypic change was detected in sepia eye colour mutant stock¹⁷. By making cross between sepia mutant with outgrowths and wild type, flies with red eye and outgrowths were obtained and a separate stock of red-eyed flies with outgrowths was established, which was used in mating propensity tests.

In mating propensity tests, multiple-choice method was used and mating success was studied by direct observation in an Elens-Wattiaux mating chamber kept in a room maintained at approximately 24°C under normal laboratory light conditions between 7 and 11 AM. Virgin females and males were collected from both the stocks and flies were aged for seven days. In multiple-choice experiment, 15 flies of each sex were used and five trials were run. Fifteen females of each of the two stocks were introduced into the mating chamber with 15 males of each of the two strains and were

SCIENTIFIC CORRESPONDENCE

Table 1. Number of matings in 60 min in the mating chamber containing 15 flies of each sex from outgrowth mutant (OG) and wild type (TD) *Drosophila bipectinata* in multiple-choice experiment

Replicates	OG ♀ × OG ♂	TD ♀ × TD ♂	TD ♀ × OG ♂	OG ♀ × TD ♂	Isolation estimate	χ^2
1	4	5	2	4		
2	6	6	2	5		
3	2	7	2	1		
4	7	6	2	4		
5	3	5	1	3		
Total	22	29	9	17	0.51	8.12*

*Significant ($P < 0.01$).

Table 2. χ^2 for 1:1 ratios on marginal totals to assess the relative mating propensities of wild type (TD) and outgrowth (OG) flies of both the sexes of *Drosophila bipectinata*

	♂	TD	OG	Total
♀	TD	29	9	38
	OG	17	22	39
	Total	46	31	
χ^2 TD, OG ♀	0.00012	$P > 0.05$		
χ^2 TD, OG ♂	2.94	$P > 0.05$		

observed for 60 min. When a pair commenced mating, it was aspirated out and the type of mating individuals was recorded. Total number of flies in each replicate was 60, and sex ratio was 1 female: 1 male.

Table 1 shows the results of multiple-choice experiment involving two strains of *D. bipectinata*. In all the replicates homogamic matings (mating between the female and male of the same type) are more frequent than heterogamic matings (mating between female and male of different types). Under the assumption of random mating, the difference between the number of homogamic and heterogamic matings was tested by calculating χ^2 . For the pooled data, the χ^2 value of 8.12 shows significant difference ($P < 0.01$) between homogamic and heterogamic matings. Thus there is a significant deviation from random mating which shows preferential (non-random or assortative) mating within *D. bipectinata*. The females of one strain prefer their own males and discriminate against alien males. The value of isolation estimate is also low (0.51), indicating sexual isolation

between two types of flies. Table 2 shows the χ^2 values calculated on marginal totals to assess the relative mating propensity of two sexes of both strains. Both types of females are equally receptive. However, wild type males are more successful in mating than mutant males but the difference is not significant statistically. Thus there is no difference in mating propensity of two types of flies.

It is evident from the present results that the thoracic outgrowths in *D. bipectinata* affect mate-recognition system, leading to behavioural reproductive isolation.

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Inhibition of filarial proteases by antibodies from human filariasis

Lymphatic filariasis caused by *Wuchereria bancrofti* is a major public health disease in India. Epidemiological studies indicate that people living in endemic regions acquired resistance to the disease after prolonged exposure to infection¹, although the direct evidence for immunity in human has been difficult to establish. Probably protective immune reactions to the parasite would be multifactorial—comprising both non-specific and specific (immunological) effector components. Elucidation of natural immune mechanisms operative in endemic population will help in devising better control strategies against the disease. Immunochemical characterization of antigens, especially those having biochemical functions (e.g. enzymes), can provide data relevant to the biology of parasites. Proteases of parasites are an important group of enzymes which are actively involved in various aspects of host-parasite interactions², for example in parasite nutrition, inactivation of host immune response, and invasion of host tissues. Antibodies inhibitory to proteases were detected in animals immune to or infected with the parasites^{3,4}, thereby emphasizing their importance. Antibody-mediated inhibition of protease activities may induce arrested growth of parasites and consequently benefit the host. Indeed, proteases have been implicated in conferring resistance to many diseases like malaria⁵, trypanosomiasis⁶, dictyocaulosis⁴ and others^{7,8}.

Studies on immunological role of filarial proteases in endemic people have been initiated by us recently. They are described as allergen⁹ and immunodiagnostic antigen¹⁰ in *W. bancrofti*-infected individuals. Host inhibitory antibody response to filarial proteases, especially in humans, has remained unknown. Here we report the presence of such antibodies in filariasis and relate their generation to the severity of infection. The effect of antibodies isolated from different groups of filarial sera was studied on the protease activities in *W. bancrofti* infective larvae (L₃) that initiates human infection, adult extract (AE) of *Setaria digitata*, an immunoanalogue of human parasite and a protease fraction (Fr. III) purified from *Setaria*.

Filarial sera were collected from disease-free normal (endemic normals, EN) and infected individuals (symptomatic

Table 1. Antibody-mediated inhibition of filarial protease activity

Filarial group	Control activity remaining (%)		
	<i>S. digitata</i> (AE)	<i>W. bancrofti</i> L ₃	Fr. III
Endemic normals (EN)	100	100	100
Asymptomatic microfilaraemic (AS)	60	65	100
Chronic filariasis (CP)	30	0	0

Protein A-sepharose purified antibody (60 µg) from filarial sera was incubated with parasitic extract (10–20 µg protein) prior to azocoll hydrolysis. The extent of inhibition was expressed as the percentage activity remaining relative to a control without IgG. The means of two independent assays are shown.

chronic patients, CP and asymptomatic microfilaraemics, AS) living in a filarial endemic village (Olosingh, Khurda district, Orissa). Pooled sera of different groups of filariasis were made by adding an equal volume of serum from individuals ($n=20$ in each group). A control serum pool (NEN) was also prepared from residents of Koraput (non-filarial region) district of Orissa. IgG was purified from each serum pool by protein A-sepharose column.

Saline soluble homogenate of *W. bancrofti* L₃, *Setaria digitata* adult (AE) and an allergenic fraction with protease activity (Fr. III) were prepared as described before^{10,11}. Protease activity was measured using azocoll¹⁰, a general protease substrate. Samples (20 µl) were pre-incubated for 1 h at 37°C and 4 h at 4°C with IgG from filarial or non-endemic normal sera in 100 µl of assay buffer before performing protease assay with azocoll.

As shown in Table 1, IgG from chronic patients was the most effective in inhibiting protease activities in all preparations. IgG from AS sera partially inhibited activities in *W. bancrofti* L₃ and AE but not in Fr. III, indicating differences in antigenicity of the protease. IgG from EN and NEN (data not shown) sera was not inhibitory to the proteases.

These results indicate that neutralizing antibodies to filarial proteases were generated during natural course of human filariasis. The generation of inhibitory antibodies depends on the severity of infection. Thus chronic patients with elephantiasis and hydrocele have highest level whereas endemic normals, a group exposed to infection but non-infected, have undetectable level of these antibodies. Sera from asymptomatic microfilaraemic individuals, another infected

group in filariasis, showed intermediate degree of inhibition. It would be worthwhile to find out what triggers the generation of such protease-neutralizing antibodies during filariasis—is it related to pathological changes or immune effector mechanisms.

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Contribution of fluid mechanics to recent developments in meteorology

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A number of general fluid dynamical, mathematical and computational questions are considered in this review relating to numerical weather predictions, namely discretization of the governing partial differential equations whose solutions on length scales smaller than that of the discretization have repeatable and characteristic (or 'eigen') elements; optimum use of data in incompletely posed problems; combining statistical and deterministic solutions, and the development of objective methods for choosing between patterns according to certain criteria. Finally the contributions of fluid dynamic and meteorological concepts as aids to human forecasters are emphasized.

THE most important application of the study of meteorology, and meteorological fluid mechanics, is the improvement of forecasting of weather and climate.

In the last century and even today people question whether forecasting is a scientific activity. This problem was raised officially in the Board of Trade and Royal Society report on the Meteorological Office in 1866, which had been founded in 1854, and was issuing two day forecast in the *Times* newspaper in the 1860s. The strictures of the report led to a cessation of forecasts for 12 years from December 6th, 1866! (It is thought that the enquiry leading to this report contributed to the depression and suicide, in 1865, of the pioneering, first director of the Meteorological Office, Vice-Admiral Fitzroy.) Taking a more optimistic view, L. F. Richardson, working at the Meteorological Office in 1913, was so sure of the value of a scientific approach that he reflected whether one day forecasting would 'resemble the process by which the Nautical Almanac is produced'. However, as we learnt later from the research on chaotic dynamical systems by E. N. Lorenz¹ in the 1960s, forecasting can never be that accurate, however elaborate the calculations or comprehensive the data on which they are based. In fact, scientific forecasting follows quite closely the principles set out in Richardson's 1922 book *Weather Prediction by Numerical Process*².

Since forecasting began, it has involved physical and fluid dynamical concepts. It still does so; but increasingly it involves mathematical and computational modelling of atmospheric processes by using the results of physical

science, particularly fluid dynamics and thermodynamics. More recently, the chemical processes in the atmosphere have also begun to be modelled with a special emphasis on their long term effects on the stratosphere, especially concentrated within the polar vortices where as fluid mechanics has shown, the horizontal diffusion can be quite weak³.

Principles and problems of numerical meteorological forecasting

The essential procedure for forecasting the atmospheric environment by numerical methods, first set out by Richardson, is (a) to divide the atmosphere into flat grid 'boxes' (that are very shallow near the ground and deep in the stratosphere), (b) using the differential equations of dynamics and thermodynamics to estimate average values of physical quantities (such as pressure and velocity) within these boxes, and (c) using the equations again; this time the equations are used to calculate how these average quantities change with time, which, like space, is divided into discrete intervals. Neither the steps (b) and (c) are exact procedures and so, not surprisingly, even at this advanced stage of numerical weather prediction, there are significant variations in how these approximations are made by different research teams (e.g. the effect on momentum transfer of deep convection⁴).

The methods used by meteorologists for calculating the effects on the larger scale 'resolved' motion of atmospheric processes on the subgrid scale are quite different to those currently used in the numerical simulation of turbulent flows, such as those applied to problems in engineering. Rather than assuming that these motions are simply unstructured chaotic eddying, an

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essentially different assumption is made that within each flat box whose horizontal extent Δx is a few tens of kilometres wide and whose depth (l) ranges from a few hundreds of metres up to a few kilometres, there is a single *type* of atmospheric motion generally having a defined observational characteristic area and internal structure (e.g. particular forms of velocity, humidity and temperature properties dependent on l). This assumption is obviously not valid across weather fronts where there are steep gradients that vary in horizontal and vertical directions. Then, although the model smooths the changes across the front between the states of the atmosphere either side of it, the model does predict these changes and the mean movement and distortion of the front. However, sometimes this lack of *small scale* discrimination leads to *large scale* errors, e.g. in the form of 'cut-off' vortices around low pressure regions.

The secondary assumptions are that there are only a finite number of 'types' and that the 'types' can be defined by the values of the quantities calculated on the scale of the grid box (see Figure 1). For example, convection patterns and cloud types are found to be determined by the degree to which the local mean density and velocity gradients of the resolved scale motions are stable or unstable to small disturbances. This is approximately defined by the Richardson number, $Ri = g\Delta\rho l / \Delta u^2 \rho_0$ (ref. 5), where $\Delta\rho$ and Δu are the changes in velocity and potential density across the box.

In the language of mathematical physics one might say that atmospheric motions on the scale less than Δx have a small number of eigen-states; or in the language of turbulence specialists, 'coherent structures'.

I believe that the first person to make this bold hypothesis was Luke Howard⁶, who stated:

If clouds were the mere result of the condensation of vapour in the masses of atmosphere which they occupy, if their variations were produced by the movements of the atmosphere alone, then indeed might

the study of them be deemed an useless pursuit of shadows, an attempt to describe forms which, being the sport of winds, must be ever varying, and therefore not to be defined.

But however the erroneous admission of this opinion may have operated to prevent attention to them, the case is not so with clouds. They are subject to certain distinct modifications, produced by the general causes which effect all the variations of the atmosphere; they are commonly as good visible indications of the operation of these causes, as is the countenance of the state of a person's mind or body.

This provided the rationale of this classification of clouds that meteorologists have followed ever since. Modern meteorology agrees with Howard in attributing the physical causes to the clouds' forms. But whereas he thought clouds caused their own electrostatic fields, it is now known that they set up their own fluid- and thermo-dynamical fields; that is where science has progressed!

The numerical procedure of deriving equations that only account for the motions of the atmosphere above a certain length scale and time interval can also be performed by representing these motions and other phenomena using the 'spectral technique', in terms of sinusoidal waves – following⁷. The problems of 'averaging out' the smaller scales are similar in the two methods.

In mathematical terms, the problem has now been converted from one of calculus (i.e. solving differential equations) into a very large algebraic calculation, which, as Richardson pointed out, is rather like turning the clock back from Newton, Leibnitz and Bernoulli's analyses of curves by calculus, to Archimedes' analysis by segments!

However, even if the equations describing the behaviour of the atmosphere (at least averaging within each grid box) were exactly correct, the results could not represent the actual state of the atmosphere unless there

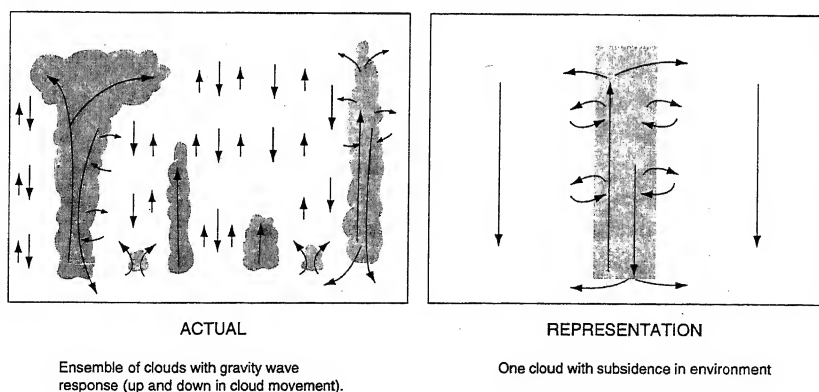


Figure 1.

were sufficient data, at a given time, to begin the calculations. Note that the data introduced into the simulations are point measurements; they contain only a fraction of the information about the local atmosphere recorded by a meteorological observer (cloud types at different heights, visibilities in different directions, etc.). One of the reasons why the recently developed techniques of 'data assimilation' enable the forecast calculations to make optimum use of the atmospheric and oceanic data is that, for each stage, the data is inserted in such a way that locally the atmosphere is close to a dynamic balance (otherwise a new data point would produce spurious accelerations⁸). This type of calculation, where the data and the methods are both imperfectly specified, is derived from mathematical control theory. It is beginning to be applied to engineering fluid mechanics, based on similar principles, in predicting the movement of turbulent eddies in order to control the flow by small active wall elements⁹.

Once the procedures are defined, the next general question is how to make best use of computers. The obvious choice is to reduce the size of the grid 'boxes' and the time intervals over which the atmospheric behaviour is averaged (the relative magnitudes of these boxes and intervals being approximately determined by the speed and time variation of atmospheric waves, weather patterns and the thermal radiation balance). Other choices are to increase the complexity of numerical modelling (e.g. of advection schemes) and of physical modelling, to improve the accuracy with which measurements are analysed (especially the very costly data from satellites that need to be utilized more effectively) and to spend more computer time on 'data assimilation' calculations to optimize the fit of the modelled atmospheric behaviour to the present and past data.

When operational numerical weather prediction began in the UK in the 1960s, the emphasis was on using computers to reduce the size of the grid boxes in the horizontal and the vertical and time interval. This was because most of the errors were caused by the large grid size, and because it was thought that most of the significant 'weather', especially clouds and rain, were determined by 'dynamic' processes and by the broad patterns of convergence and divergence of the horizontal wind field. This is largely true for the kind of conditions, particularly the frontal systems which dominate weather at high latitudes.

But it is not a valid assumption for weather that is strongly affected by local ground heating/cooling and evaporation, such as those occurring in continental climates, and even in the UK in summer!

Consequently, the present practice is to use increased computer capacity to improve the physical modelling and data analysis, rather more than reducing the grid-boxes' size.

Different ranges of forecasting weather and climate

Different types of calculations and data use are necessary for forecasts over short periods, and for distances that are small compared with the scale of the globe. For specific local calculations, for example wind at an airport or wind energy over a hill, the horizontal size of some 'boxes' may be as small as a few metres. However, for daily forecasts, at least three Meteorological Services in Europe now calculate atmospheric conditions on the mesoscale of 10–15 km. This kind of calculation leads to significant improvements in the forecasts of rainfall patterns, especially where there are strong topographic effects. The next step forward will include assimilation of data from weather radars and satellites, and, a real fluid mechanical challenge, more detailed flow field models, including '1½' level turbulence models.

Over a longer period, beyond the 5 days of the usual deterministic global forecasts (on a resolution of 90 km), it is found that, when slightly different data are fed into the calculation, on some occasions a number of quite different weather patterns are forecast; but in other circumstances, all the forecast weather patterns remain quite similar to each other. (This is the nearest that weather forecasting has come to make use of 'chaos' theory in practice¹⁰.) In the latter situations, the forecast beyond 5 days should be more reliable. The study of these sets of forecasts may be a way of estimating the reliability of longer range forecasts, a method being investigated in detail at the European Centre for Medium Range Weather Forecasts at Reading, as well as in the UK and US Meteorological Services. These and other services are collaborating to find the best practical use of these 'ensemble forecasts' and, just as important, the best way to communicate their results to the public and customers, possibly by a greater emphasis on making forecasts in probabilistic terms. Some Weather Services consider that these ensemble forecasts provide more useful information for improving extended range forecasts over 6 to 10 days than is provided by the information obtained with a single forecast based on reduced grid size. The greater the number of realizations in these ensemble forecasts, the greater computer time required. Despite the fact that they have to be run with larger and fewer grid boxes, the usefulness of this approach is now widely recognized.

A promising modification to these large number of 'deterministic' calculations from given data (whether actual or with some slight artificial differences) is to make forecasts, of, say, temperature beyond 5 days ahead, by only using computations of the large scale behaviour of the atmosphere, such as whether there will be a static high pressure region or a sequence of depressions carried by strong westerlies. Then this in-

formation is combined with statistical information obtained over previous years that link these weather systems with the local information required by the forecasts such as temperature and rainfall. This 'mixed' approach is proving to be more accurate than simply relying on average climatology for forecasts of up to 1 month ahead. It is valuable to certain commercial customers who have to make business decisions based on estimates of the weather 10 to 30 days ahead. Decisions based on computer modelling techniques can benefit most from these long range statistical forecasts. However, the results are of limited use to the general public who would find it difficult to make use of a forecast that is reasonably correct only above 7 times out of 10! (Note that this is a significant achievement, because if average climate statistics only were used, the accuracy would be about 50% (ref. 11).)

In some parts of the world – particularly the Sahel in Africa and NE Brazil – the good statistical correlation between the climate and the slow variations of the temperature of the upper layers of the ocean enables the UKMO to provide satisfactory seasonal forecasts of rain and temperature, that local Met. Services and government agencies find very valuable. (A critical fluid dynamic problem here is to improve the modelling of the depth of the mixed layer of the ocean, which depends on estimating the entrainment processes between a well mixed turbulent region above the 'thermocline inversion' and the stratified region below.)

The methods used for numerical weather prediction over a few days are also being applied to the numerical prediction of 'climate'. This may be broadly defined, as the statistics of the average state of the atmosphere and oceans over many years, or more precisely on the statistics defined over this period, such as the mean and extreme values of rainfall, temperature, etc. Thus, since the weather in any given month varies from year to year, the 'climate' of a country for July is determined by averaging over several years. Therefore, by definition, changes in 'climate' can only be detected by considering how the average, over say 10 years, changes from one decade to the next. It cannot be detected by comparing one or two years with each other. It follows that numerical predictions of climate are also based on calculations over many years, and therefore are not 'forecasts' in the conventional sense; no-one can predict the weather in July 2094, but it might be possible to predict the changes in climate, averaged over a decade, one hundred years from now.

In these numerical models for the prediction of climate, space is also divided up into grid boxes within which the motions are calculated over specific intervals of time. Since, even beyond a month or two, the variation in sea-surface temperature affects the atmosphere, it is clear that climate forecast models must include the

behaviour of the oceans. Calculations using a 3-dimensional coupled ocean-atmosphere model require the largest computer systems in the world. There are only 4 such installations at present, one being at the Hadley Centre of the Meteorological Office. Even these computer installations, running full tilt for a few months, only have the capacity to provide climate forecasts for periods of the order of 100 years. A major application of these models has been to forecast how average global temperatures are likely to rise over the next century as a result of increasing concentrations of carbon dioxide and other greenhouse gases in the atmosphere. By allowing for aerosols in the atmosphere, the calculations have to some extent been validated by their reasonably close agreement with the observed trends of average global warming.

Such a model was run by Hansen¹² in the USA to estimate the effects on global temperature of the aerosols caused by the eruption of Mt Pinatubo in 1991. It was predicted in early 1992 that there would be a decrease by about 0.5°C in 1992 and a subsequent rise of about 0.2–0.3°C in 1993, although these values require some correction to allow for the effects of the El Nino Southern Oscillation in the Pacific Ocean. Since both predictions have been borne out by the measurements, this demonstrates that certain features of the global climate are consistent with forecasts. Because 3-dimensional coupled atmosphere-ocean model calculations take so long to perform, computer models have been devised for exploring the effects of many different influences on the global climate. These include very long term effects of variations of the radiation emitted by the sun, or wobbles in the earth's orbit about the sun, both of which lead to seasonal fluctuations in the solar radiations reaching the earth ranging from 2% to 10%. In these models, the behaviour of the atmosphere around the whole surface of the earth at each height is averaged; in the mathematical analysis the atmosphere is divided up, not into boxes, but into concentric skins (like that of an onion). Instead of a million boxes there are now about one hundred layers. Then the state of the atmosphere is calculated in each layer (or onion skin). It has been possible to run such '1-dimensional' models over periods of 10,000 years or longer and simulate many of the climate changes of the ice ages, which have been discovered from the analysis of gases trapped in bubbles found in ice cores.

Developments

How will forecasting improve over the next few years? There has been a steady increase in the accuracy of forecasts produced by mathematical/computational models being 'fed' by data from atmospheric measurements, and an even greater increase where they are used in

conjunction with human intervention (which I return to later). The accuracy of the 72-hour forecast now (measured in terms of root mean square error of the surface pressure) is about the same as the 24-hour forecast less than 15 years ago. It is expected that the present 24-hour forecast error (of about 2 hPa) will be reduced by at least 10% over the next 5 years (Figure 2).

There are three main reasons why further improvements will come about; firstly, research is leading to new insights into atmospheric processes and their representations in numerical models, and into the best way that data may be inserted into the models. For example, through fluid dynamic studies at turbulent inversion layers, more precise criteria are being developed to determine whether stratocumulus cloud grows in thickness, which leads to rain, and drizzle, or on the other hand, breaks up (because the turbulent eddies gain enough energy by entraining dry air from above the cloud to cause local cooling and thence sink with increasing speed), leading, of course, to clear skies. This fluid mechanics research also leads to a better knowledge of the sensitivity of the forecast, which also has to be communicated! Entrainment processes and up/downdrafts in deep clouds are also of critical importance for modelling their overall structure and internal energetic eddies. Those driven by the cooling of evaporating droplets can contribute significantly to the strength of the gusts at the ground. It is now realized that these may cause the highest gust speeds that produce sudden wind forces on structures, disrupting or destroying surface transport systems; the spatial forms of these cloud-driven gusts tend to be significantly different from those produced by boundary layer shear which we simulated in wind tunnels^{11,13}.

Once such local processes are understood sufficiently well to be modelled, their net effect has to be averaged or 'parameterized' over the grid boxes in the forecast calculations, an aspect of model development that requires some ingenuity and usually some empirical adjustment to obtain the best results. The modelling of atmospheric

flow over terrain and its effect on the terrain drag on the flow over a grid box is an active area of research¹⁴. Recent fluid dynamics research on turbulent flow over undulations and on stably stratified flow is now being incorporated into forecast models. An important development is to combine different types within one grid box; precipitating ice cloud above hill stratus can greatly increase the rainfall rate compared to stratus alone.

The effectiveness of such changes for the performance of the models has to be examined carefully with statistical techniques, as described by Hollingsworth¹⁵. Only if the sizes of the grid boxes are reduced below the scale of the characteristic motions can the processes be modelled explicitly. That is not yet practical. For small reductions in the size of the grid box, the parameterizations appropriate for each process have to be adapted to the new size because the physical processes, such as lee waves over mountains, have their own particular length scales.

Since forecasts require, in principle, updates of data in every grid box, the second way to improve forecasts is by providing data in those grid boxes where data is currently unavailable, especially 3 to 30 km above the ground at the altitude of the dominant dynamical processes. Remote sounding instruments on geostationary (at 36,000 km above the earth) and polar orbiting satellites (of which there are two at any time, each pass overhead twice a day at an altitude 850 km) measure radiation in the visible, infra-red and microwave frequency bands.

In 1993 the European experimental polar orbiting satellite ERS-1 produced global data of near surface wind speeds, by measuring scattered microwave radiation from ocean waves; the variation of the wind over several kilometres is related to the wind structure above the surface layer and has contributed to a 10% improvement in the model's accuracy over data sparse areas such as the Southern Pacific.

An excellent example of the use of satellite images in combination with computers and human operation is the forecasting of the trajectories of tropical cyclones.

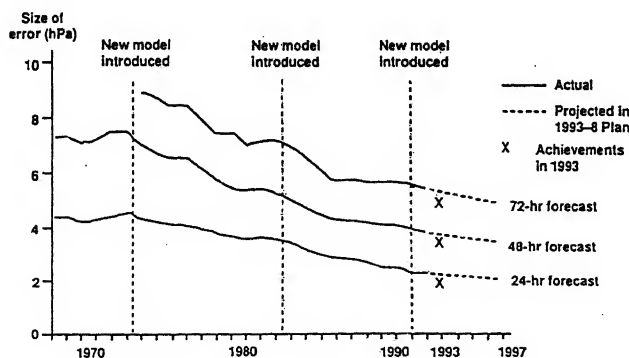


Figure 2. Forecast errors: 1967–1992, actual; 1993–1997, projected.

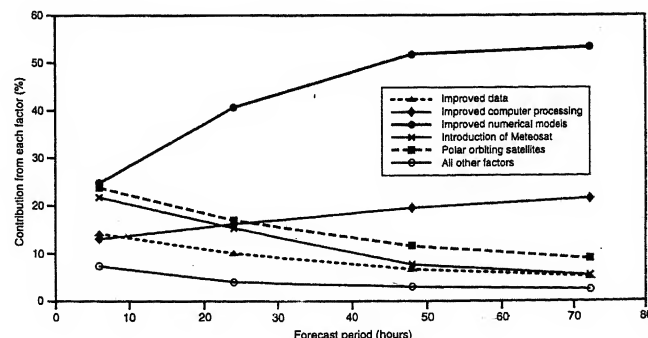


Figure 3. Estimates of the contribution of different scientific and technological factors to the improvement in forecast accuracy.

Many of these cyclones form in remote areas where no other measurements are available, and are only detected once they have become strong enough to organize clouds into patterns that are visible via satellites. They are not always well predicted by the computer models because initially they are too small, their diameter (about 100 km) being similar to the size of the grid box of the model. After spotting a cyclone, or perhaps learning about it from other sources, a special technique is currently necessary. The human, or 'intervention', forecaster introduces into the numerical code a simple mathematical representation of the vortex to represent the central regions of the cyclone. Then its track is computed¹⁶, a particularly critical task because its direction often changes by 90° in 24 hours. A significant (30%–40%) reduction in the errors in these forecasts has recently resulted from improving the model of the 'vortex' to allow for its asymmetry and history¹⁷. More elaborate models are now under development, especially in the USA, to represent on small grids moving with the cyclone the detailed winds and precipitation within them, and thence to reduce the errors of freak forecasts. Because of the devastating effects of the cyclones, warnings based on meteorological forecasts are widely publicized on TV and other media. Actions based on these warnings by the emergency services and the public are now saving thousands of lives annually around the world.

The third element in the improvement of forecasts is the provision of greater computer capacity. In the UK Meteorological Office there has been an increased speed of calculation, from around 2000 Floating Point operations per second in 1960 on the Mercury computer to around 6 gigaflops on the current CRAY C90. Every ten years over the past 40 years the power of the computer has increased by a factor of 60, even though the individual processor elements have increased by a smaller factor (of about 10) (ref. 18). At the same time smaller 'workstations' (about 1/100 of the power of the large systems) have improved by a factor of 30 over the past decade, so that these can now be used for many of the research studies and, even local forecasts. Additional power will continue to result from increasing the number of integrated circuits within each silicon 'chip', and from steady improvements in how these chips are connected, housed and cooled within the computers – i.e. its 'architecture'.

In the conventional vector-processing computers only a few calculations (about 8 on the CRAY YMP-8) can be performed simultaneously, and all data are stored in a central shared memory. The next major step in computer architecture in meteorological forecasting has been the introduction in 1995 of massively parallel processors (MPP). A key feature of MPPs is that memory is distributed with the processors. For the first time the

architecture of the computer will correspond more closely to the nature of the processes in the atmosphere. Massively parallel computers will allow calculations to be performed at many grid points (about 100 or more) in the computer simultaneously. This MPP architecture (which is less expensive than conventional vector processors for a given level of power) should permit continued refinement of present forecast models for several further generations of computers. Although the most efficient use of this approach requires programs to be adapted specifically for this new architecture, the form of these new programmes is common to many scientific disciplines (e.g. meteorology, aeronautics, hydraulics, etc). Extensive collaboration is now under way across Europe in all these different fields of application of computational methods.

Two different advanced features of numerical weather prediction will be helped by these improvements in computers. First, instead of simply updating the calculation as new data arrives (or 'nudging' the solution), there will be greater emphasis on repeating the original calculations to fit the subsequent data more smoothly⁸. There will be many iterative cycles of calculation in this 'data assimilation' procedure which will give improved predictions by making better use of the previous data. Secondly, there will be time for the calculation of ensembles of different atmospheric states to provide the basis for the mixed dynamical and statistical methods of longer range forecasts, and to estimate their reliability.

So far there has been little discussion of the present and future role of the human forecaster. Until recently, s/he has been interpreting measurements and qualitative observations very much along the lines developed by V & J Bjerknes' Bergen School¹⁹, firstly identifying typical synoptic features and types of air mass, and then with the aid of Sutcliffe's²⁰ concepts, estimating their movement and development by inspection. These and more recent conceptual developments (e.g. the use of potential vorticity at different levels) aid the understanding and the correction of the numerical models. Interpretation of the computer output is a *scientific procedure* in which hypotheses are made and subsequently, of course, tested. It is based on a knowledge of the data that was introduced into the model calculations and of the characteristics of the one or more models that are available to the particular forecaster. It is found that skillful intervention by the forecaster often leads to a better result for the customer than is obtained by relying on the prediction of any one model, especially in those situations when the various model predictions differ. In some synoptic situations, numerical models are very sensitive to small perturbations. A classic case, that is repeated every few months, is the 'extending frontal trough', which may or may not disrupt into cut-off low pressure systems. The forecaster has to use

and interpret all the extra evidence available to him/her that is not in the model (e.g. details of cloud types, detailed humidity profiles, potential vorticity plots, etc.) to make a prediction. The forecaster has to make best use of his/her experience, including making an assessment of how the different models work in the particular synoptic conditions, and give emphasis to different aspects according to the special needs of the various 'customers' of the forecasts.

Some aspects of the human forecaster's contribution could be supplanted by 'knowledge-based' systems developed from the human experience. However, to replace the continual learning by the forecaster would first require the development of a complex self-learning 'neural-network' system. Although no one has yet attempted to do this at the synoptic level, this approach is being adopted for very short range forecasts (less than 3 hours) based mainly on interpreting radar and satellite images. In general, the human forecaster's contribution, although no longer dominant, will remain very important for the foreseeable future.

The number of users and uses of meteorological forecasts are steadily increasing. For example, forecasts are being used for an ever wider range of activities by members of the public, while aviation forecasts, which continue to be vital for the safety of those flying, are now required with greater precision for economical flight planning. Then there are the many specialized uses of forecasts in the retail industry, the oil industry, the public utilities and so on. See, for example, ref. 21. Forecasts are also needed in scientific studies of the atmosphere, and particularly in field experiments in the rapidly growing activity of environmental forecasting. The latter include the dispersion of pollution, wind forces affecting structural safety, wind energy, generation of sea waves, precipitation and flooding, environmental transport problems, and changes in the chemical composition of the atmosphere. Each of these uses requires different information about the atmosphere. As Bjerknes, who began as a fluid dynamicist, found when he was developing his ideas, new applications play a valuable

role in testing and improving all aspects of meteorological and climate forecasting.

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Planetary motions and the birth of classical mechanics*

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The subject matter of this article straddles the birth of modern science and involves several larger-than-life figures – Nicolas Copernicus (1473–1543), Tycho Brahe (1546–1601), Galileo Galilei (1564–1642), Johannes Kepler (1571–1630) and Isaac Newton (1642–1727). This account will be woven in a connected and coherent way around their lives and work. Copernicus came first in this group; then Brahe, Galileo and Kepler overlapped and were in contact with one another; and after they were all gone came Newton.

MATHEMATICS, medicine and astronomy are the oldest areas of knowledge in all civilizations. In each culture there has evolved some model of the universe, some 'system of the world', as a framework within which to fit astronomical observations. For our present purpose we are concerned with the so-called medieval model. Before describing it, let us briefly look at some of the essential details about planets and their orbits (Table 1).

From ancient times till near the end of the 18th century – and this includes the period from Copernicus to Newton – only the earth and five other planets were known.

The medieval model was a combination of Greek science going back to Aristotle and Ptolemy, and later Christian theology. Figure 1 is a picture of the universe as presented by Dante Alighieri in the 13th century in his *Divine Comedy*¹. The earth is a globe at the centre. Then there are nine solid translucent crystal spheres, each centred on the earth. The first seven revolve and transport respectively the Moon, Mercury, Venus, Sun, Mars, Jupiter and Saturn in sequence. The eighth sphere carries the fixed stars. Last of all comes the ninth sphere, the Primum Mobile, which like an engine drives all the others. Beyond that is heaven, the home of God. In Aristotle's version, there were actual spheres, while later Ptolemy replaced them by circular orbits. This emphasis on spheres and circles reflected the feeling that these were the most perfect geometrical figures. There was also a sharp distinction between terrestrial

matter and phenomena, and celestial ones. In this picture the heavens were perfect and unchanging.

The medieval model of the universe slowly gave way under advances in astronomy and mathematics, over the 16th and 17th centuries. The principal blows were struck by Copernicus, Tycho Brahe, Galileo and Kepler. Let us at this point recall briefly the life and work of each of them².

Copernicus was born in 1473 in Torun in Poland. He stands at the boundary – the transition point – between medieval and modern science. For a ten-year period, from age 24 to 34, he studied in Italy at Bologna, Padua and Ferrara. He was very close to his teacher, Domenico Maria da Novara, the relationship between them being as between a guru and a shishya. Copernicus studied mathematics, astronomy, philosophy, law, medicine and theology – the times seem long gone since one could even contemplate such an endeavour. He also had direct access to Greek sources. During his sojourn in Italy, Copernicus saw the need to replace the Ptolemaic system by a simpler one in which calculations would be easier. His ideas were elaborated much later and led to his book *De Revolutionibus Orbium Celestium* (*On the Revolution of the Celestial Spheres*). He retained the notion that celestial bodies travel on circles lying on uniformly rotating spheres; in any case the data available to him was not accurate enough to show up departures from circular motions. Figure 2 shows a picture of his model of the universe³. The sun, rather than the earth, was at the centre. Then followed in sequence the spheres carrying Mercury, Venus, the earth, Mars, Jupiter, Saturn and the fixed stars. This heliocentric model had been suggested by Aristarchus in 300 BC, and Copernicus rediscovered it. In his words, as the sun is the visible form of God, 'in the centre of everything the sun must reside; in the most beautiful temple created by God, there is the place which awaits him where he can give light to all the planets'⁴.

Copernicus showed mathematically that this model could work and was simpler than Ptolemy's. However for fear of censure he presented it cautiously as a hypothesis, useful only for calculations. He also spoke of the concept of relative motions, with respect to the sun or the earth regarded as stationary; and showed that the earth both rotates on its own axis and revolves

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SPECIAL SECTION: PERSPECTIVES IN ASTRONOMY

Table 1. Planets and orbits

Planet	Mean distance from sun (million miles)	Revolution period	Number of satellites	Remarks
Mercury	36	88 days	0	Known since antiquity
Venus	67	225 days	0	"
Earth	93	1 year	1	—
Mars	142	1 year 322 days	2	"
Jupiter	483	11.9 years	16	"
Saturn	866	29.5 years	18	"
Uranus	1783	84 years	15	Discovered 1781 by Herschel in England
Neptune	2794	165 years	8	Predicted 1843–46 by Adams/Le Verrier; discovered 1846 in Berlin
Pluto	3666	249 years	1	Discovered 1930 by C. W. Tombaugh in USA

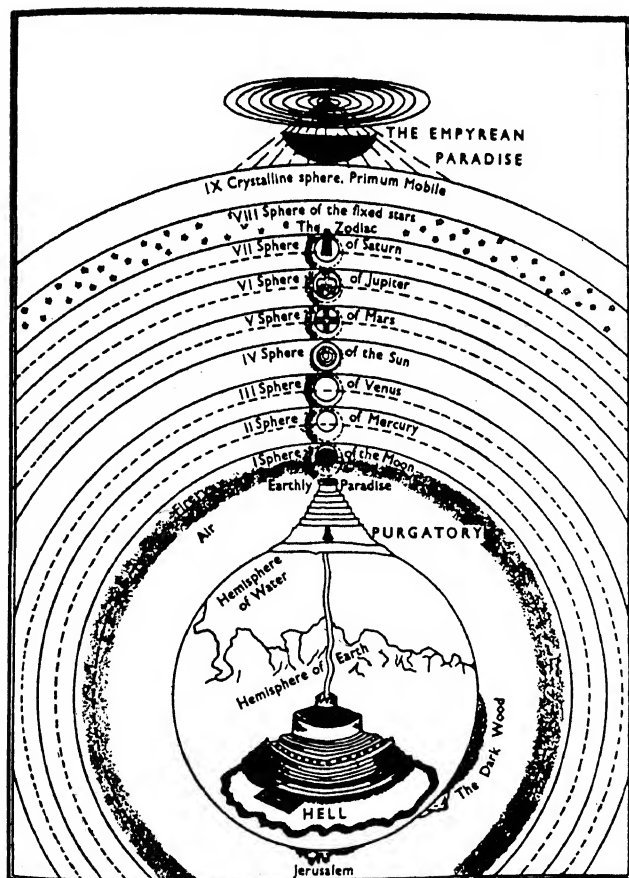


Figure 1. The medieval model of the universe, from Dante's *Divine Comedy*, 13th century.

about the sun. His book was technical and difficult for the average reader; it took some fifty years for his ideas to spread. Giordano Bruno, Galileo and Kepler were

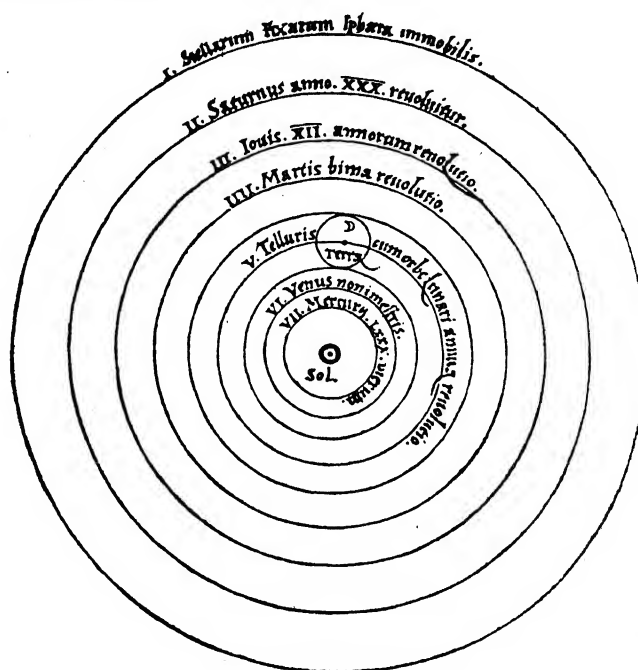


Figure 2. The Copernican model of the universe, 1543 AD.

among the few who understood and accepted Copernicus' ideas. Bruno paid with his life in 1600; while Galileo and Kepler reinforced each other's beliefs privately, before each publicly acknowledged the new system.

Brahe came from the Swedish nobility, and was interested in astronomy and astrology. He was also a very gifted instrument maker and observational astronomer. The King of Denmark supported him handsomely to set up a fine observatory on the island of Hveen. Here he designed his own new instruments, and kept daily astronomical records meticulously for many years. His figures are accurate to within 1' or 2' of arc, about

one-sixtieth of a degree. (Recall that these were the days before optical telescopes.) This later became a treasure for Kepler to work on.

Two major discoveries of his made it clear to Tycho that the heavens are not immutable, that celestial bodies are changeable: one was the appearance and disappearance of a star in the years 1572–1574, another the passage of a comet beyond the moon in 1577. However, he did not accept the Copernican model but had a hybrid of his own – the sun and the moon circled the earth, while the other five planets circled the sun. For ease in calculations, this stood midway between the Ptolemaic and the Copernican systems.

Around 1599, due to changed fortunes, Brahe left Denmark and moved to Prague as 'Imperial Mathematician' to the Emperor Rudolph II. Kepler joined him there in January 1600, but Brahe died soon after in November 1601. Galileo admired Brahe's instruments and data but not his theories. Ultimately Brahe's data together with the Copernican model percolated to others and set the stage for major advances by Kepler and Galileo.

The lives and stories of Kepler and Galileo overlap a great deal. For convenience let us look first at Kepler and then turn to Galileo, making some comparisons as we go along. Kepler was a student of theology in Tübingen, gifted in mathematics and possessing mystical tendencies. In contrast, Galileo was led much more strongly by the power of reason and experiment. From his teacher Michael Maestlin at Tübingen, Kepler learned privately of the Copernican model. In 1594 he was obliged to leave for Graz as a high school mathematics teacher. Here while in class he had a flash of insight revealing to him a new geometrical model for planetary orbits with the sun at the centre. There were precisely six planets because there were exactly five perfect solids, and these were nested in successive spheres. The solids in sequence are the cube, the tetrahedron, the dodecahedron, the icosahedron and the octahedron. The innermost sphere lay inside the cube, touching its faces; the next one passed through its vertices, but touched the faces of the tetrahedron; and so on. The fit to the radii of planetary orbits was almost, but not quite, perfect. He wrote out his ideas, mixed with much speculation, in his book *Mysterium Cosmographicum* published in 1596, and sent copies to Galileo, Brahe and others. In this book he publicly declared support to the Copernican model – Galileo did so much later, in 1613.

The agreement of his model with the data was almost perfect. Kepler saw that to make progress much more accurate data was needed, and only Tycho had it. Kepler wrote to Tycho for help and, as it happened, simultaneously the latter invited the former to join him at Prague as his assistant. This was in 1600. Tycho had been much impressed by Kepler's work, and wanted

him to help vindicate his own hybrid model. Soon after, when Tycho died in November 1601, Kepler ended up inheriting all the precious data (notwithstanding problems with Tycho's son-in-law Tegnager).

Now Kepler set to work at a feverish pace; he did enormous calculations to improve his model, working particularly on the troublesome orbit of Mars. The period 1601 to 1619 – over which his three laws of planetary motions were discovered – was one of high drama. In brief the main steps he took were: (a) he created the true heliocentric model by placing the sun at the 'centre' of planetary orbits, modifying Copernicus' procedure of placing the geometric centre of the earth's orbit there; (b) he found that each planetary orbit lies in a plane containing the sun; (c) the orbit of Mars, which proved the most difficult, almost fitted a circle except for two data points; (d) as a result, with Tycho's accurate data, he saw that a circular orbit for Mars was not possible; (e) he also gave up the assumption of uniform speed for Mars as it traverses its orbit, allowing a speeding up or slowing down when close to or far away from the sun; (f) from the data he then discovered the Law of Areas: the radius vector from the sun to Mars swept out equal areas in equal intervals of time; (g) and again from the data he found at last that Mars moved on an ellipse with the sun at a focus. These two laws discovered in the order mentioned during 1602 were announced seven years later in the 'Astronomia Nova' but in the opposite order: the Law of the Ellipse became the First Law, and the Law of Areas the Second. Imagine waiting for seven years today before announcing laws of such magnitude!

After dealing with Mars, Kepler showed that these two Laws hold for the earth too, and then extrapolated them to all the other planets. The Third Law, which links different planets to one another, took much more work; a true search for harmony in the heavens, it was announced in 1619 in the 'Harmonice Mundi'. It states that the periods of revolution are proportional to the $3/2$ powers of the mean distances from the sun. This became the key to the discovery of the inverse square law of gravitation by Newton and others. Added to all this, Kepler came very close to the law of gravity. He saw the sun – and not angels with flapping wings – as the agent keeping the planets on their courses. But he unfortunately died before Galileo's delayed publication, in 1632, of his experimental studies on the laws of motion, especially the laws of inertia and of uniformly accelerated motion.

Galileo chose physical science in preference to medicine which his father had wanted him to pursue. He was highly respected as a mathematician at the University of Padua. Whereas in his correspondence with Kepler he supported the Copernican model, till as late as 1606 in his lectures he taught the medieval model. Only in

1613, as mentioned earlier, did he publicly express support for the Copernican view. His 'change of heart' came from his discoveries in astronomy.

During the period 1589–1591 at Pisa, his researches were mainly concerned with the description of motion: falling bodies, inclined planes, uniform acceleration. (Here we may parenthetically remark that it took Galileo several years to arrive at the concept of acceleration which to us today seems so obvious and natural!) Thanks to his work, 'for the first time, all the important features of motion – distance covered, speed, and acceleration – were expressed in terms of time. Galileo had realized that time is the independent variable in the description of motion; indeed, he was the first to time physical events. This was an extremely fruitful idea... it implied an entirely new conceptual view of the world, codified by Isaac Newton forty eight years later'. Then, in 1609, he heard that a telescope had been invented in Holland. He promptly reinvented it, and achieved even greater power. Then he turned his telescope to the heavens – and changed astronomy for ever!

Galileo saw that the moon was scarred, with mountains and valleys, hardly a perfect sphere; he found sunspots which moved. In 1610 he found four satellites circling Jupiter, all coplanar and the nearer ones circling faster. So everything in the sky did not circle the earth! And just as Jupiter with its circling moons went round the sun, so too our earth with our moon could orbit the sun. Again in 1610 he discovered the phases of Venus, just like our moon. So Venus like the earth was dark, went round the sun, and reflected the sun's light. He found moons around Saturn too, and almost saw the rings. Here was telling evidence for the Copernican theory, and the immutability of the heavens had been destroyed.

We all know the trouble Galileo got into with the Church upon expressing his views in his 1632 treatise *Dialogue on the two Chief World Systems, the Ptolemaic and the Copernican*. After this historic controversy and confrontation, he returned to his earlier studies on the science of motion, and published his results in *Discourses Concerning two New Sciences*. This is the first great modern work on the kinematics of motion. Smuggled out of Italy in 1638 and published in Holland, the message spread. To some degree Galileo was continuing an existing tradition of study of mechanics, created by the Schoolmen of the Universities of Oxford and Paris in the fourteenth century. John Buridan – probably better known for his ass – had already created the 'impetus' concept. But much he made himself, especially in showing that Aristotle's ideas about motion were untenable.

As precursors to Newton, and as the giants on whose shoulders he stood, Kepler and Galileo left him a splendid legacy – the laws of planetary motion, and the laws of mechanics. Newton synthesized and systematized

all this, discovered the Law of Universal Gravitation which unified terrestrial and celestial phenomena, and went far ahead in many ways. He accomplished the most decisive transition from description to explanation of natural phenomena.

Already around 1665 at age twenty three, Newton had conceived of the universal inverse square law of gravity, and seen that it could explain both the fall of the apple on the earth, and the moon's going round the earth. As he recalled many years later: 'I deduced that the forces w^{ch} keep the Planets in their Orbs must be reciprocally as the squares of their distances from the centers about w^{ch} they revolve: & thereby compared the force requisite to keep the Moon in her Orb with the force of gravity at the surface of the earth, & found them answer pretty nearly'⁵. But he could not proceed further for two reasons. One was the rather inaccurate data available to him at that time. The other was a major mathematical problem which he solved only in 1685: the proof that with an inverse square law of force, and no other, a uniform spherical mass acts as though all its mass were concentrated at its centre.

Now we come to the famous 1684 visit of Edmund Halley to Newton in Cambridge⁶. In January of that year, at a discussion among Halley, Christopher Wren and Robert Hooke at the Royal Society, the question arose of deriving *all* of Kepler's Laws from the principles of dynamics. Many had realized that this was the key problem whose solution was crucial for further progress. By combining Kepler's Third Law with a recent formula of Huyghens for the centripetal force, Halley had concluded that the force of attraction between sun and planet must be proportional to the inverse square of the distance between them. And he was not the only one to have found this – so had Wren and Hooke too. But Kepler had anticipated it, and Newton had used it in 1665. At the Royal Society discussion, Hooke claimed he could conversely derive all of Kepler's Laws from the inverse square law of gravity; but he would not divulge the details till the others had tried and failed. While Wren doubted Hooke's claim, Halley admitted he was unable to do this. All in all the stage was set for Halley's visit to Newton in August 1684. What transpired is best told in Newton's own reflections recorded by de Moivre:

'In 1684 Dr Halley came to visit him in Cambridge, after they had been some time together, the Dr asked him what he thought the Curve would be that would be described by the Planets supposing the force of attraction towards the sun to be reciprocal to the square of their distance from it. Sir Isaac replied immediately that it would be an Ellipsis, the Doctor struck with joy and amazement asked him how he knew it, why saith he I have calculated it, whereupon Dr Halley asked him for his calculation without any farther delay, Sir Isaac

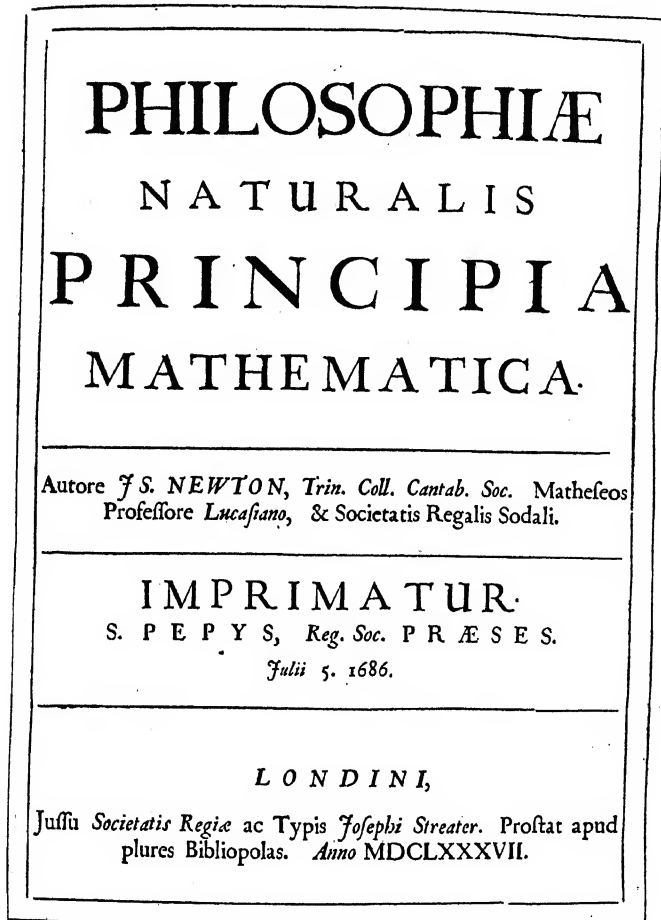


Figure 3. Title page of Newton's *Principia*, 1686 AD.

looked among his papers but could not find it, but he promised him to renew it, & then to send it him....'

As Westfall comments, Newton was being cagey in saying he had misplaced his work—he too wanted to be careful and not let others see his calculations! The missing papers have since been found. Anyway this was enough indication for Halley to persuade Newton to publish his findings so that all the world could see what he had accomplished. This became a herculean effort lasting three years and resulting in the publication, over 1686–1687, of the '*Philosophiæ Naturalis Principia Mathematica*'—*Mathematical Principles of Natural Philosophy*—the greatest book on science ever written⁷. The title page is shown in Figure 3. It was like creating a new language with its own grammar, and writing an epic poem in it, all at once! In the *Principia*, Newton laid the foundations of classical mechanics in an axiomatic and deductive form à la Euclid, and created the framework for progress in the succeeding centuries. He picked up from Galileo's work on mechanics, and extended, systematized and completed it, all at once.

Then he applied it to his law of universal gravitation and showed how all of Kepler's Laws followed. He demonstrated that a mathematical model of natural phenomena, involving observed and measured quantities and possessing predictive power, could be built.

Newton's First Law of Motion was a restatement of Galileo's principle of inertia. Even Descartes had said that an isolated body moves in a straight line. The Second Law of Motion had been grasped by Galileo. The Third Law—the equality of action and reaction—was Newton's own and unique contribution. It became the basis and set the pattern for Conservation Principles. All this was presented against a background of clearly stated views on the natures of space and time.

The Law of Gravity as we saw had been foreseen by Kepler, but the unification of the terrestrial with the celestial was Newton's achievement. He showed that the moons of Jupiter and of Saturn going around their parent planets obey Kepler's Laws; the planets going around the sun do so; the moon round the earth does likewise. Everywhere throughout the solar system his principles of mechanics and gravity held sway. His theory for cometary orbits went beyond Kepler, and he explained the tides and many another phenomenon.

That is the story of planetary motions and the birth of classical mechanics. Newton's methods were propagated mainly in Europe by Euler, Lagrange, Laplace and others. And success followed success. One stunning episode from the nineteenth century is worth brief mention, and that is the story of Neptune. Around 1820 there remained some disagreements between theory and observations on the orbit of Uranus, and Bessel suggested there might be another planet causing disturbances. (Of course in Kepler's time the mutual influences of the planets upon one another were not accessible, but much water had flowed under the bridge since then.) In 1843 a student at St. John's College in Cambridge, John Couch Adams, completed a dissertation on this problem and calculated the orbit of the suspected new planet in detail. In 1845 he alerted the Greenwich Observatory to look for it. Independently, Urbain Jean Le Verrier of Paris showed in June 1846 that the data on Uranus could not be explained without the presence of another planet about twice as far away. He sent his prediction to the Berlin Observatory. Finally success came at Berlin: on two successive nights, September 23rd and 24th of 1846, the planet Neptune was spotted just where the theorists had predicted it would be!

Later in the century, discrepancies arose between theory and observations for the precession of the perihelion of mercury. After accounting for all known perturbing effects, there remained an unexplained precession amounting to 43" of arc per century. Faith in Newton's programme was so strong that it was assumed that yet another planet was at work—and it was named Vulcan!

But there is no Vulcan, and that problem was solved in an entirely unexpected way by Einstein's general theory of relativity. That theory gave a language to talk about a much larger world than that of the Ancients or of Newton's World System—but that, as the bartender said to Irma, is another story.

Suggestions for further reading, references and footnotes

The theme of this presentation has been so well studied for so long that excellent sources are available in plenty. Only a very select set of possible references will therefore be presented here.

1. This figure is taken from Hanbury-Brown, R., *The Wisdom of*

Science – its relevance to Culture and Religion, Cambridge University Press, 1986, p. 43.

2. Giorgio Abetti, *The History of Astronomy*, Sidgwick and Jackson, London, 1954; Arthur Berry, *A Short History of Astronomy*, Dover, New York, 1961; Mathews, P. M., 'Galileo and Kepler – Precursors of Newton', in *Proceedings of the Seminar on '300 Years of Newton's Principia'* (eds Shrivastava, S. K. and Mukunda, N.), ISRO-IISc Space Technology Cell, Indian Institute of Science, Bangalore, 1986.
3. Taken from Brown-Hanbury, R. ref. 1 above, p. 46.
4. Abetti, G. ref. 2 above, p. 70.
5. Richard S. Westfall, *The Life of Isaac Newton*, Cambridge University Press, 1993, p. 39.
6. Richard S. Westfall, ref. 5 above, ch. 8.
7. In this connection, see the highly interesting account given by D. Speiser, 'Newton's Principia', CERN Report 80-02, 25th February 1980, CERN, Geneva, Switzerland.

Origin of the highest energy cosmic rays*

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After briefly recalling the history of discovery of cosmic rays, the present status of the subject is discussed. Cosmic-ray particles with energy exceeding 10^{20} eV have been detected. The origin of cosmic rays remains an unsolved problem in physics and astrophysics. The nature of the source(s) as well as the physical mechanism(s) responsible for endowing the cosmic-ray particles with extremely high energies are not known with certainty. We discuss some recent ideas in this context with special emphasis on the problem of origin of the highest-energy cosmic rays.

1. A brief history of cosmic rays

The story of the discovery of cosmic rays forms one of the most fascinating episodes of the history of science in the very early part of the current century. It is hard to associate the discovery of cosmic rays entirely with any one single experiment. Indeed, a number of remarkable experiments performed by a number of adventurous physicists, many of whom were tantalizingly close to the 'discovery', preceded the actual announcement of the discovery. A nice account of this history is given in the book by Pomerantz¹ which forms the basis of the historical aspects of the subject described in this section.

The Austrian physicist Victor Hess announced his discovery of 'an extra-terrestrial source of penetrating

radiation' in 1912 after a series of heroic balloon-borne experiments performed by him over the previous one year. The 'penetrating radiation' was later christened 'cosmic rays' by Robert Millikan in 1926. The word *extra terrestrial* is important here, for it was well known at the time of Hess's experiments that our own Earth is also a source of 'penetrating radiation' due to natural radioactivity of soil and various rocks.

Natural radioactivity was the 'in' physics in the closing years of the nineteenth century and the early years of the twentieth century. Within a year of the discovery of X-rays by Röntgen in 1895, Becquerel discovered natural radioactivity (in 1896). Even before the discovery of radioactivity, experiments with gold-leaf electroscope, an instrument that could measure the presence of free electric charges (i.e. ionization) in a medium, and which played a major role in the history of discovery of cosmic rays, showed presence of leakage currents associated with ionization in sealed containers even in apparent absence of any obvious ionizing source; the leakage current seemed to correspond to an average rate of formation of ions of ~ 10 ions/cm³/sec. This ionization was naturally attributed to the presence of 'radioactive material' in air and in soil. As revealed by later experiments, this conclusion was largely correct, but not entirely so!

In 1898 Rutherford established two different kinds of radioactive emissions: (i) α -rays and (ii) β -rays. The α -rays were found to be of high ionizing capacity, easily absorbable in media (range \sim few cm), and were later identified as the nuclei of ^4He atoms. The β -rays, in

*Invited talk presented at the Symposium on 'Interface of Astronomy with other Sciences', organized by Indian National Science Academy and held at the Indian Institute of Astrophysics Observatory, Kodaikanal, on 4-5 May 1995.

contrast, were more penetrating, and were quickly identified as electrons. In 1900 Villard discovered γ -rays, which were found to be very penetrating. The γ -rays suffered no deviation in external magnetic fields, which revealed their electrically neutral nature. (It was much later, in 1914, that Rutherford and Andrade were able to identify γ -rays as highly energetic electromagnetic radiation by observing reflection of γ -rays from crystal surfaces.)

In the meantime, experiments performed on the ground as well as underground continued to show the presence of leakage currents associated with ionization. Physicists like C. T. R. Wilson, E. Rutherford and many others were involved in these experiments. McClennan and others performed experiments on sea. In 1910 the Italian physicist Pacini made detailed analyses of the data of several earlier ground-based as well as sea-based measurements of residual ionization of air, and concluded that it was hard to attribute the observed residual ionization entirely to the known radioactive substances.

Was the observed residual ionization caused entirely by Earth's natural radioactivity? To settle this question an obvious experiment to do would be to measure the level of ionization of air high up in the atmosphere where the effects due to Earth's radioactivity should diminish. Such an experiment was performed by the German Jesuit Fr. Wulf in 1910. He measured a rate of ionization of ~ 6 ions/cm³/sec at ground level at Paris, and about 64% of this value when measured on top of the Eiffel Tower (~ 300 m above the ground). At the time of Wulf's experiment, γ -rays were the most penetrating form of radiation known, and any ionization associated with Earth's radioactivity at such a height was expected to be essentially due to γ -rays. However, Wulf's measured value of the rate of ionization on top of the Eiffel Tower was about 6.4 times more than what was expected on the basis of the then known attenuation properties of γ -rays in air. On the basis of these measurements Wulf concluded that there might exist another γ -ray source in the upper atmosphere(!), or, that the γ -ray absorption coefficient in air might be actually smaller than the then not-so-well known value. In retrospect, we thus see that Wulf was indeed very close to 'discovering' the cosmic rays.

It was, however, left for Hess to take the crucial step of measuring the variation of ionization *as a function of altitude*. Hess's experiments consisted of carrying a charged electroscope in an open gondola flown by a balloon filled with hydrogen – physically a rather dangerous experiment! Hess attained a height of about 1000 m in 1911 and finally ascended to about 5300 m in the following year. What Hess found was that the ionization inside his sealed electroscope first decreased with altitude, but then started to increase beyond a height of ~ 800 m, and continued to increase steadily to the highest altitudes

attained by him. Hess also made separate measurements of γ -ray absorption in air using an intense radium source which showed that γ -rays from ground were completely absorbed at a height of ~ 500 m from the ground. Hess's analysis of his data was unambiguous – it pointed towards the '... presence at great altitudes of previously unknown matter or ... of an extra-terrestrial source of penetrating radiation', a discovery for which Hess was awarded the Nobel Prize in 1936. A balloon ascent to a height of 9300 m by the German physicist Kolhörster in 1914 confirmed Hess's conclusions. The outbreak of the World War I in 1914 stopped further balloon flights until around 1922 when Robert Millikan and his coworkers in the USA undertook further balloon-borne experiments.

Millikan's initial results seemed to be contradictory to Hess's. In fact, in 1924, Millikan announced that there were 'no such penetrating radiation'. But in 1926, after a series of careful measurements of the ionization level over snow-fed lakes at different altitudes above the sea level, Millikan was finally convinced that 'very hard ethereal rays of cosmic origin were entering the earth uniformly from all directions'. Together with G. H. Cameron, Millikan went one step further; based on the prevailing (wrong!) notion at the time, that the 'penetrating radiation' responsible for the ionization of air consisted of very high-energy γ -rays, and by extrapolating the known penetrating power of low-energy γ -rays to very high energies, they tried to derive the spectrum of these cosmic ' γ -rays'. Their derived ' γ -ray' spectrum seemed to consist of discrete monoenergetic components corresponding to the binding energies of nuclei of some common elements like C, N, O, Na, Mg, etc. This, together with the fact that the process of formation of any element (starting with hydrogen as the basic element) would be expected to be associated with release of the nuclear binding energy corresponding to that element, led Millikan to conclude that cosmic rays represented 'the birth cries' of elements in 'the depths of space'. Indeed, because of this supposed 'cosmic' connection, Millikan referred to the penetrating radiation as 'cosmic rays'. In retrospect, we now know that the assumption of Millikan and Cameron that cosmic rays were γ -rays, was wrong, and so was their conclusion about the implied relationship of cosmic rays with element formation process.

We now know that cosmic rays are mostly electrically charged particles, *not* γ -rays. This came about with the discovery of the so-called latitude effect, published in 1927 by the Dutch physicist J. Clay, according to which, the cosmic ray intensity was systematically less near the equator than at higher latitudes. This could be adequately explained only if the cosmic-ray particles were assumed to be electrically charged particles. For in that case, the particles, being of extraterrestrial origin, would encounter greater difficulty in reaching the Earth's

surface near the equator where the Earth's magnetic field lines are nearly parallel to the surface, than they would near the poles where the magnetic field lines are almost vertical.

In 1930s the distinction between the *primary* and the *secondary* cosmic rays was established. The primary cosmic rays (consisting mostly of protons, and with a lesser abundance, nuclei of other heavier elements like helium, carbon, nitrogen, etc.), striking the top of Earth's atmosphere from outside at relativistic velocities interact with the air atoms in the upper atmosphere and generate a lot of other secondary particles. Indeed, the residual ionizations measured in the early experiments that led to the discovery of cosmic rays were mostly due to the secondary (and higher generation) electrically charged particles originating from the interaction of primary cosmic-ray particles in the upper atmosphere. The primary cosmic-ray particles can be directly measured only by experiments at those high altitudes at which there is practically no residual atmosphere above the detector, which correspond to heights (above the sea level) of ~ 40 km and above. Knowledge about the primary cosmic rays has been obtained by means of these high-altitude balloon-borne (and, of course, unmanned!) experiments. Satellites and space probes have also added to our knowledge of the spectrum and composition of the primary cosmic rays.

In the next section we briefly review our current knowledge of some of the general characteristics of the observed cosmic rays, in particular, their energy spectrum, flux, and composition. There are a large number of reviews and monographs on cosmic rays giving details. For a recent review and references, see, Drury². In §3 we discuss the question of origin of cosmic rays, highlighting the problems posed by recent experimental results on the extremely high energy cosmic rays (having energies in excess of 10^{20} eV). In §4 we discuss the recent idea of their having a fundamentally different origin (*vis-à-vis* the lower-energy cosmic rays) in the sense that they may not be produced by any astrophysical acceleration mechanisms (that are believed to be responsible for producing the lower energy cosmic-ray particles) but rather by some non-acceleration process, one example of the latter being production of energetic particles from decay of massive particles released in the process of collapse or annihilation of cosmic topological defects like monopoles, cosmic strings, etc. We summarize our discussions in §5.

2. General characteristics of the observed cosmic rays: Composition and energy-spectrum

As mentioned above, the cosmic rays that reach the Earth's surface are the byproducts of interactions of the

primary cosmic rays (that strike the top of Earth's atmosphere) with the air nuclei. In the following, by cosmic rays we will always mean the primary cosmic-ray particles unless explicitly stated otherwise.

The cosmic rays are mainly composed of nuclei of common elements such as H, He, C, Fe and so on, constituting almost 98% of the total composition; the rest are mainly electrons. There is also a small percentage of positrons and antiprotons, which are mostly, if not entirely, of secondary origin resulting from interactions of the galactic cosmic-ray particles with the interstellar medium. The composition of cosmic rays is different in different ranges of energy. Up to $\sim 10^{12}$ eV, the nuclear component (often referred to as the 'hadronic' component) of cosmic rays is dominated by protons (p) (about 87%), followed by the He nuclei (α) (about 12%), and heavier nuclei like those of C, Fe, etc. (about 1%). The mean abundances of various elements in cosmic rays are roughly the same as the average 'cosmic abundances' of these elements, although there are important differences for certain elements; these differences are, however, well-understood in terms of spallation of some of the heavier nuclei into lighter nuclei in collision with the material in the interstellar space. At TeV (10^{12} eV) and PeV (10^{15} eV) energies, the composition is roughly 50% protons, 25% α -particles, 13% C, N, O and 13% Fe. The measurement of the composition at higher energies becomes increasingly difficult since the flux of cosmic rays at energies above $\sim 10^{15}$ eV is so low that direct measurements are not possible—one has to take recourse to methods such as extensive air-shower techniques (see below).

The kinetic energy of the hadronic component ranges from about 0.1 GeV per nucleon ($1 \text{ GeV} = 10^9 \text{ eV}$) to extremely high energies $\sim 10^{20}$ eV and more per nucleus. (The composition of cosmic rays at the highest energies is not known with certainty; so the measured energy at high energies is usually given in terms of energy per nucleus as opposed to energy per nucleon used at low energies where the composition is known.) Between $\sim 1 \text{ GeV}$ and $\sim 10^{15}$ eV, the differential energy spectrum of the hadronic component is roughly a power-law, $\propto E^{-\alpha}$, with α lying between roughly 2.5 and 2.7. Below about 0.1 GeV, the spectrum drops off sharply because the magnetic field carried by the solar wind blowing out from the Sun sweeps these low-energy particles away from the inner solar system, thereby reducing the intensity of these low-energy particles on Earth significantly. This phenomenon is called 'solar modulation', which is stronger at sunspot maximum than at sunspot minimum.

At about 10^{15} eV, the spectrum steepens to a power-law with $\alpha \sim 3.2$. This is believed to be due to relatively large (compared to the thickness of the galactic disk) gyro-radius of the lighter cosmic-ray particles in the

$\sim 10^{-6}$ G magnetic field of our Galaxy. In other words, the galactic magnetic field is unable to contain the more energetic but lighter cosmic-ray particles (protons) within the galactic disk. Thus the overall flux decreases and at the same time the composition of cosmic rays changes towards a relatively higher abundance of heavier nuclei for which the magnetic containment within the Galaxy is relatively more effective, the gyro-radius being smaller for a heavier particle. Above an energy of about 10^{18} eV, the spectrum changes slope again, this time becoming less steep, with $\alpha \sim 2.6$ again. Recent measurements seem to indicate that the composition changes back to dominance of lighter particles over heavier ones. These 'ultra high-energy' (UHE) particles are so energetic that the galactic magnetic field would be too weak to contain them within the Galaxy, and therefore, these particles are believed to be mostly of extragalactic origin. This cosmic-ray spectrum is now known to extend to at least $\sim 3 \times 10^{20}$ eV. This is almost a macroscopic amount of energy, all of which is essentially in the form of kinetic energy. In other words, the particles have been somehow accelerated to speeds almost that of light. Where do these UHE cosmic-ray particles come from and how do they attain such high energies? These remain some of the unresolved issues in physics and astrophysics of cosmic rays. We shall return to these issues in the next two sections. For the moment, we continue with our discussion of the general characteristics of the observed cosmic rays.

The integral particle flux in cosmic rays, i.e. the total flux of particles above a given energy E , is about 1 particle/cm²/sec at $E \sim 10^{10}$ eV; about 1 particle/m²/year at $E \sim 10^{17}$ eV; and about 1 particle/km²/century at the highest energies. The particle flux is thus a steeply falling function of energy (see Figure 1). Nevertheless, since the particles themselves are very energetic, the total energy density in the form of cosmic rays in the universe is ~ 1 eV/cm³, which is comparable to, e.g. the total energy density in the form of starlight (~ 0.6 eV/cm³), or that in the form of galactic magnetic field (~ 0.2 eV/cm³). Thus cosmic rays constitute an important component of the total energy budget of the Universe.

Since the intensity of cosmic rays is a steeply falling function of energy, it becomes increasingly difficult to measure directly the charge and energy of cosmic rays as energy increases. Indeed, the particle flux of cosmic rays at energies above about 10^{15} eV is so low that balloon- and satellite-borne experiments become impractical; they have too little effective detector area to capture sufficient number of cosmic ray events for measuring the spectrum. Fortunately, at high energies, another method becomes available. This is the method of extensive air shower (EAS), discovered by the French physicist Auger and his group in the 1930s. When a

high-energy primary cosmic-ray particle (say, a proton) strikes an air atom (more specifically, say, a nitrogen or an oxygen atom) in the atmosphere, it creates a lot of secondary particles through high-energy nuclear interactions. The energetic secondary particles, in turn, further interact with other air atoms and generate tertiary particles, and so on, until the energies of the created particles fall below the threshold for the relevant multiparticle production processes. The high-energy multiparticle production processes are typically 'forward' processes, i.e. the daughter particles are produced with their momentum vectors confined within a narrow cone whose axis lies along the momentum vector of the parent high-energy particle. Moreover, the daughter particles all travel with more or less the same relativistic speed. Thus, at any time, the particles' positions lie on a thin disk, which thus propagates down the atmosphere with relativistic speed and can even reach sea level, typically if the energy of the primary particle exceeds $\sim 10^{15}$ eV. The 'disk', whose radius can be several hundred metres, can contain several millions of particles (mostly electrons and photons) depending on the energy of the primary particle that initiates the shower. The shower particles can be sampled and detected by an array of particle detectors placed several metres apart on the ground, each detector covering an area of a few square metres.

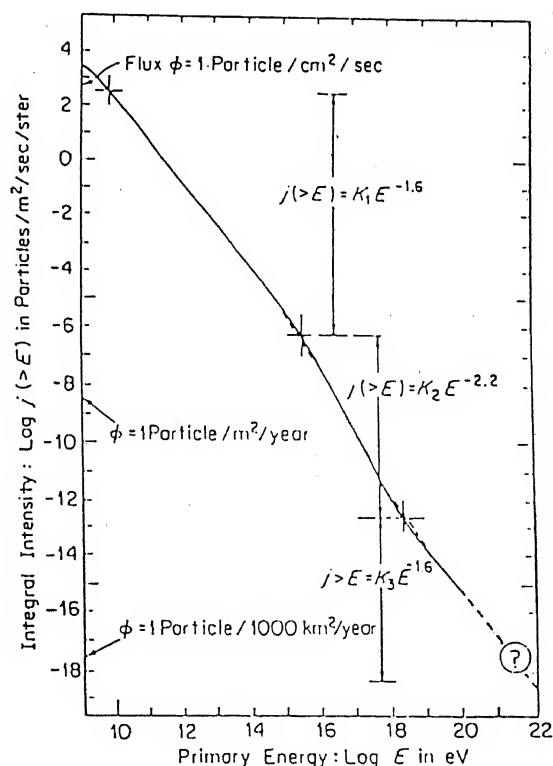


Figure 1. Integral energy spectrum of primary cosmic rays (from ref. 1).

(The total area covered by the array can, in principle, be as large as desired, say, hundreds or even thousands of square kilometers, although there are practical problems in building very large arrays.) From the measured difference in the arrival times of the shower particles at different locations on the ground, the inclination of the disk axis to the vertical direction can be worked out, thereby giving information on the arrival direction of the primary particle. The energy of the shower-initiating primary particle can be worked out from the measured profile of the density of the shower particles on the ground.

The method of EAS has been employed by various experimental groups to arrive at the spectrum of cosmic rays to extremely high energies, exceeding 10^{20} eV (refs 3–10). The filled circles in Figure 3, for example, represent the so-called UHE (i.e. with energy $E \geq 10^{18}$ eV) cosmic-ray spectrum as measured by one of the experiments, namely, the Fly's Eye experiment⁷. Similar spectra have been obtained by other experiments (see refs 3, 4 for more details). These experiments show that the cosmic-ray spectrum does indeed extend to such high energies (albeit with steeply falling intensity as energy increases) *without any indication of a upper cut-off in energy in the spectrum*, at least up to the highest energy ($E \sim 3 \times 10^{20}$ eV) detected so far for a cosmic-ray particle. This has important implications for possible sources and the underlying acceleration mechanisms responsible for producing these extremely high-energy particles, which we shall discuss in the next two sections. The composition of these extremely high-energy cosmic ray particles is also not well determined. However, recent results of one of the experiments, namely, the Fly's Eye experiment⁷ indicate that the composition is dominated by heavy nuclei (most probably Fe) from energy $E \sim 10^{17}$ eV to $E \sim 5 \times 10^{18}$ eV at which there is a dip in the spectrum accompanied by a change of composition from 'heavy' to 'light' (probably proton, or, possibly even photon).

3. Origin of cosmic rays

The question of origin of cosmic rays consists of two distinct yet interrelated questions: (a) what powers the 'engine' that endows the cosmic-ray particles with the enormous energies that some of them evidently possess? (b) what is (are) the source(s) of these particles? The conventional view is that origin of cosmic rays is linked to some of the most energetic phenomena in the universe represented by active objects like supernova explosions, pulsars, quasars, active galactic nuclei, radio galaxies, and so on. The consensus seems to be that cosmic rays with energy at least up to $\sim 10^{15}$ eV are of galactic origin, i.e. their sources reside within our Galaxy. However, the charged cosmic-ray particles on their way to earth from a given source are repeatedly scattered and

bent by the non-uniform magnetic field of our Galaxy. There is thus, in general, no simple relationship between the arrival direction of the particle at earth and the original direction in which it is ejected from the source. It is, therefore, not possible to trace the source(s) of these cosmic rays to any specific object(s) in the sky. Nevertheless, it is possible to gauge the nature of the potential sources from simple considerations of energetics: The power output of any candidate source must be larger than the power represented by the observed flux of cosmic rays, a rough estimate of the latter being $\sim 10^{34}$ W (see ref. 2 and the references therein). It is widely believed that cosmic rays up to an energy of $\sim 10^{15}$ eV are linked with supernova explosions. The arguments are as follows: A typical supernova explosion releases $\sim 10^{44}$ J of mechanical energy; on the average one supernova explosion occurs in a typical galaxy in about every 30 years (although none has occurred in our own Milky Way since Kepler's supernova of 1604!). Thus the power output in a typical supernova is $\sim 10^{35}$ W. Therefore, a supernova origin of the bulk of cosmic rays requires that roughly about 10% of the energy output of the supernova be used in some kind of acceleration process that boosts low-energy charged particles to the very high energies observed in cosmic rays.

How exactly the acceleration process might work is a subject of considerable complexity and is beyond the scope of this article. Very briefly, essentially all the acceleration mechanisms proposed in this context are variants of a basic idea originally due to Fermi¹¹. In Fermi's original mechanism, charged particles are accelerated by repeated collisions with moving 'magnetized clouds'; the macroscopic kinetic energy stored in the moving clouds is transferred to the particles through their repeated encounters with the clouds. Fermi's basic idea, with some important modifications, has been applied to acceleration of particles in a variety of realistic astrophysical situations, such as acceleration of particles in passing through moving shock waves generated by supernova explosions, acceleration in active galactic nuclei (AGN), in radio galaxies, and so on. A particularly successful mechanism, that is currently favoured by most theoretical cosmic-ray physicists, is the so-called 'diffusive shock acceleration mechanism' (see Drury² for a review and references to original literature). A major characteristic feature of all 'Fermi mechanisms' and, in particular, of the diffusive shock acceleration mechanism (DSAM), is that particles emerge from the acceleration site with spectra that are power-law in energy, like the observed cosmic-ray spectrum. The power-law index (α) of the spectrum is related to basically two characteristic time-scales in the problem, namely the acceleration time scale and the escape time scale, and is, therefore, calculable. The typical values of α in DSAMs satisfy $\alpha \geq 2$.

The DSAM is typically a slow acceleration process; the average energy of any particle increases gradually as the particle crosses the shock region back and forth many times. The particles are confined within the shock region by the magnetic field which, therefore, plays an important role in all variants of the Fermi mechanism. However, for a given strength of the magnetic field and for a given size of the shock region, as a particle's energy increases it becomes increasingly difficult to prevent the particle from escaping from the shock region. This prevents further acceleration of the particle. The process is therefore, self-limiting: For a given strength of the magnetic field (B) and for a given maximum size of the acceleration site (R), there is, in general, a maximum energy, E_{\max} , to which a particle of a given charge and mass can be accelerated. This severely restricts the candidate acceleration sites for the most energetic cosmic rays.

Apart from the slow Fermi acceleration mechanism, several fast or 'one shot' acceleration mechanisms have also been proposed, the chief example of which is acceleration in the induced electric field due to strong rotating magnetic field around pulsars. There are typically two major drawbacks of these kinds of fast acceleration scenarios, viz. (a) a power-law spectrum is not a characteristic feature of these acceleration mechanisms, and (b) the accelerated particles generally lose a lot of their energy in collision with particles of the dense electron-photon plasma that typically surround the high magnetic field regions around objects like pulsars.

The general relationship between the strength of the magnetic field (B) and the characteristic linear dimension of the acceleration region (R) for a variety of astrophysical objects which are suspected to be potential sites of particle acceleration is shown schematically in Figure 2. It is seen that protons or iron nuclei can, in principle, be accelerated to $E \sim 10^{20}$ eV, the most favoured candidate site for acceleration to this energy being the lobes of radio galaxies¹², although active galactic nuclei are also possible sites. Acceleration to energies significantly beyond 10^{20} eV is difficult as we run out of possible known astrophysical objects that would have the right combination of magnetic fields and linear sizes.

When numbers for B , R and the characteristic shock velocity β are put in, it is found that energies up to the 'knee' (i.e. up to energy $\sim 10^{15}$ eV) can be reasonably well achieved by acceleration in shocks associated with supernova remnants (SNRs). For higher energies, SNRs fail, and other sources must be invoked for the rest of the spectrum. As already mentioned in §2, the spectrum beyond $\sim 10^{15}$ eV is steeper than the one below it; it is also dominated by 'heavies' (probably mostly iron nuclei) up to an energy $\sim 5 \times 10^{18}$ eV at which the spectrum becomes flatter and the composition changes to 'light' particles (probably mostly protons). The steep heavy

component beyond the 'knee' and up to $\sim 5 \times 10^{18}$ eV is interpreted as a second galactic component whereas the flatter and 'lighter' component beyond $\sim 5 \times 10^{18}$ eV (i.e. the UHE spectrum) is thought to be of extragalactic origin for the reasons that the gyro-radii of these energetic particles in the galactic magnetic field would be much larger than the size of the Galaxy itself. The heavy galactic component between $\sim 10^{15}$ eV and $\sim 5 \times 10^{18}$ eV is thought to be associated with accelerations in pulsar magnetospheres. This may be because pulsars are thought to be formed in supernova explosions, and so the 'fine-tuning' problem of matching of the two components in terms of their 'amplitude' at the 'knee' may be explained in a natural way. However, the precise way this may come about remains uncertain.

The general view is that UHE component is of extragalactic origin. There are, however, stringent constraints on the distances of the possible sources of these UHE cosmic rays. A proton of energy above $\sim 5 \times 10^{17}$ eV is above the threshold for electron-positron pair creation in collision with photons of the universal 3 K thermal microwave background that is known to pervade the whole universe. Above an energy of $\sim 6 \times 10^{19}$ eV a proton in collision with a microwave background photon is even above the threshold for photo-pion production reaction. Both the above mentioned processes are significant energy-loss processes for any UHE proton propa-

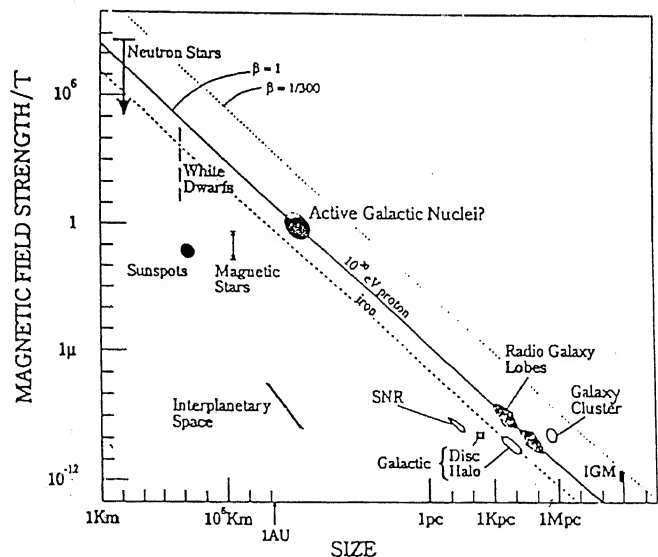


Figure 2. Magnetic field strengths and typical linear dimensions for various astronomical objects, some of which are potential sites for acceleration of cosmic rays. For illustration, the restrictions on the possible astronomical objects that can in principle accelerate protons to 10^{20} eV are indicated. Only objects on, or to the right of, the solid diagonal line can accelerate protons to 10^{20} eV for relativistic characteristic shock velocities ($\beta = 1$). The dotted line marked $\beta = 1/300$ indicates the limit if the shock velocities are only $\sim 1000 \text{ km s}^{-1}$. The applicable restrictions on the possible astronomical objects that can accelerate iron nuclei to 10^{20} eV are also indicated (from Drury²).

gating from an extragalactic source to us. The photo-pion production process is in fact a rather drastic energy-loss process for extragalactic UHE protons; it puts an upper limit to the distance of any extragalactic source from which a proton of a given energy at source can reach us with a given remaining energy. Indeed, soon after the discovery of the 3 K thermal background radiation by Penzias and Wilson in 1965, Greisen, and independently Zatsepin and Kuz'min¹³ suggested in 1966 that the UHE cosmic-ray spectrum, if it is truly extragalactic in origin, should show a cut-off at some high energy somewhere around 10^{20} eV. This predicted cut-off in the UHE cosmic-ray spectrum is now-a-days called the Greisen-Zatsepin-Kuz'min (GZK) cut-off. A similar cut-off is predicted if the UHE particles are heavy nuclei such as iron nuclei; there the relevant process is the photo-disintegration of the nuclei in collision with the 3 K photons.

Whether the measured UHE cosmic-ray spectrum indicates a true GZK cut-off remains unclear. The problem is accentuated by the recent detection of an event with $E \sim 3 \times 10^{20}$ eV by the Fly's Eye experiment^{7,8} and an event with $E \sim 2 \times 10^{20}$ eV by the Akeno experiment^{9,10}. The Haverah Park experiment⁵ as well as the Yakutsk experiment⁶ had also earlier reported events with energy $\sim 1 \times 10^{20}$ eV. It has been recently shown^{14,15} that a proton detected with an energy $\sim 3 \times 10^{20}$ eV could not have come from a source at a distance of more than about 60 Mpc ($1 \text{ Mpc} \approx 3 \times 10^{24} \text{ cm}$). Moreover, at this energy, a charged particle should not be deflected by the intergalactic and the galactic magnetic fields by more than $\sim 10^\circ$ (ref. 14). The implication is that the source of the 3×10^{20} eV event should essentially lie within a distance of about 60 Mpc from us and it should be within a cone of angular radius $\sim 10^\circ$ centered around the measured arrival direction of the event. Yet, when a source-search for this event was made in the sky^{14,15}, no potential source such as any active galactic nucleus, radio galaxy, etc. satisfying the above restrictions on the distance and positional direction was found! The highest-energy cosmic ray event, therefore, constitutes a puzzle: Here we have a particle so energetic that its essentially undeflected trajectory should allow us to more or less directly trace its sources back in the sky (unlike the case for lower energy particles which suffer random deflections due to the magnetic fields enroute). However, no suitable source is found when the trajectory is traced back!

In the next section we discuss the possibility that the highest-energy cosmic rays (HECR), i.e. the cosmic rays with energies above 10^{20} eV constitute a new component perhaps of an entirely different origin. It is even possible that the HECR particles are not connected with any acceleration process associated with any known astrophysical object, an exciting possibility that has received

a great deal of attention in recent years and to which we now turn.

4. The highest-energy cosmic rays: Possible non-acceleration origin

The existence of the HECR poses serious challenge for conventional DSAM that attempts to explain the origin of these particles in terms of accelerating them in special astrophysical situations, e.g. in relativistic shocks associated with AGNs, or in the lobes of radio galaxies. Acceleration in the latter is in principle capable of producing particles with energies as high as a few times 10^{20} eV although it requires the use of rather extreme (and perhaps somewhat unrealistic) values of parameters such as R , B , etc.

One alternative possibility is that the HECR particles have a more *fundamental* origin in the sense that they are *not accelerated* at all^{14,16-18}. Instead, these particles may simply be the decay products of some sufficiently massive elementary particle species surviving from an early cosmological epoch. One attractive realization of this idea of *non-acceleration* origin^{16,17} of HECR involves collapse and/or annihilation, in recent epochs, of cosmic topological defects (TDs)¹⁹ such as magnetic monopoles, cosmic strings, domain walls, etc., which could be formed in the early universe during phase transitions associated with spontaneous breaking of symmetries implemented in unified models of elementary particle interactions such as in Grand Unified Theories (GUTs).

Owing to their topological stability, the TDs formed in the early universe can survive down to the present epoch. The TDs nevertheless are occasionally, and in certain circumstances, frequently, destroyed in physical processes like collapse or annihilation (see ref. 16 and references therein). When a TD is physically destroyed, the energy stored in the TD is released in the form of massive quanta of the fields that 'constitute' the TD, namely, the massive gauge and higgs fields (the 'X' particles) associated with the broken symmetry. The released X particles would then decay, essentially instantaneously, typically into fundamental particles like quarks and leptons. Hadronization of the quarks would then produce jets of hadrons containing mainly light mesons (pions) together with a small fraction ($\sim 3\%$) of nucleons (protons and neutrons). The γ -rays and neutrinos from the decay of the neutral and charged pions, respectively, would thus be the most abundant particles in the final decay products of the massive X particles. If the TDs under consideration were originally formed at a GUT energy scale, the mass m_X of X particles released from the TDs can be $\sim 10^{25}$ eV. The decay of the X particles released from the TDs can thus give rise to protons, neutrons, γ -rays and neutrinos

with energies up to $\sim m_X$, very much higher than what can be achieved by astrophysical shock acceleration mechanisms. The cosmic ray particles can thus be produced directly in this scenario (referred to as 'TD model'), and no acceleration mechanism is needed.

The release of X particles from TDs may occur continually at all cosmological epochs after the formation of the TDs under consideration in the early universe. However, only the X particles released in relatively recent epochs are likely, if at all, to contribute to the present-day flux of UHE protons and γ -rays, because those released in the earlier epochs would have to traverse greater distances through the cosmic background radiation fields to reach us and would, therefore, lose significant fractions of their energy in collision with the photons of the background radiation. However, neutrinos can come from relatively earlier cosmological epochs because they suffer little energy loss as a consequence of their small cross section of interaction with the relevant particles of the background medium.

In the TD model, the shapes of the energy spectra of protons, γ -rays and neutrinos at production (i.e. the injection spectra) at any time are determined primarily by the physics of fragmentation of quarks into hadrons and not by any astrophysical parameters. At any given time, the injection spectra are, therefore, also independent of the specific kind of TDs responsible for release of X particles. Different kinds of TDs, however, evolve in different ways. Therefore, the absolute magnitude and the rate of production of the various particles, and so also the final evolved spectra, will be different for different kinds of TDs. However, in the highest energy regions, the shapes of the proton as well as γ -ray spectra become insensitive to the kind of TDs producing them, because to survive at these energies, the protons and photons must originate at relatively close (i.e. non-cosmological) distances for which the cosmological evolution is immaterial. The shape of the neutrino spectrum will, however, remain sensitive to the kind of TDs producing them and their cosmological evolution because neutrinos can propagate over large (cosmological) distance scales without much attenuation.

The injection spectra of nucleons, γ -rays, and neutrinos in the TD model have been calculated^{16,17,20} by extrapolating the available models of hadronization of quarks as described by the theory of Quantum Chromo-Dynamics (QCD) to extremely high energies. This gives approximately power-law differential injection spectra for nucleons, γ -rays and neutrinos all with a power-law index $\alpha \sim 1.3$. However, there is a great deal of uncertainty involved in extrapolating the low-energy QCD-based models of hadronization of quarks to extremely high energies involved in the present situation. It is also possible that the value of α for nucleons may be somewhat different from that for, say, γ -rays. The main

point, however, is that the injection spectra of cosmic ray particles in the TD model can, in principle, be considerably *flatter* than in the standard shock acceleration scenarios which, by and large, produce differential injection spectra with $\alpha \geq 2$.

The typical shapes of the final proton, γ -ray and neutrino spectra, including the effects due to propagation through the extragalactic medium, are shown in Figure 3. This figure was obtained for a specific TD model, namely, that involving annihilation of magnetic monopole-antimonopole pairs²¹; however, as explained above, the spectra have more or less a universal shape independent of the specific kind of TD-process one considers, especially at the highest energies, and hence the spectra shown in Figure 3 are representative of the particle spectra expected in TD models in general. One major uncertainty in this scenario is the absolute magnitude of the cosmic ray flux produced by TDs, i.e. the 'normalization' of the predicted flux. This clearly depends on the specific process of particle production involving specific kind of TDs. The normalization of the particle fluxes in Figure 3 implies a monopole abundance that is well below the stringent astrophysical

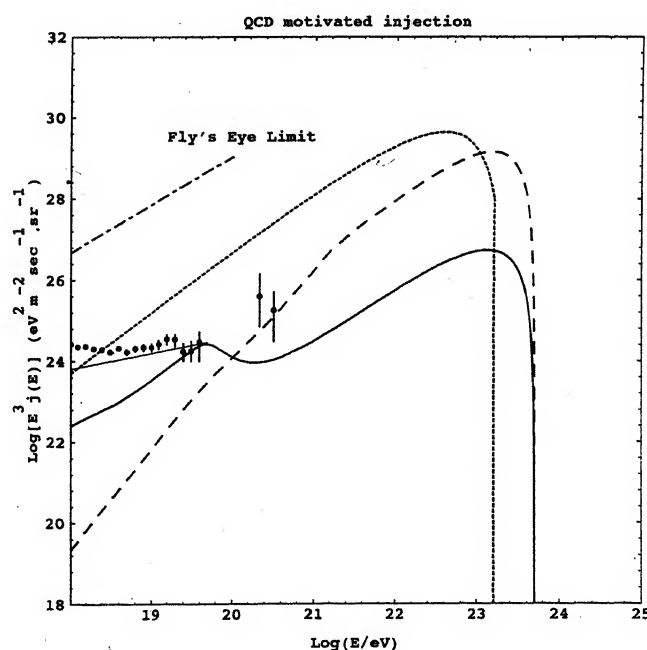


Figure 3. The proton (solid line), γ -ray (long-dashed line) and the neutrino (short-dashed line) spectra in the TD model including the effect of propagation through the cosmic background medium. The X particle mass is taken to be 10^{15} GeV and an extrapolation of QCD-based hadronization spectrum to the relevant high energies has been used to obtain the injection spectra. The combined proton and gamma ray flux has been normalized at $10^{19.7}$ eV to the 'extragalactic flux component' (thin solid line) (see ref. 7) fitted to the data from the Fly's Eye experiment (filled circles with error bars)⁷. Also shown (dash-dotted line) is an approximate limit on the neutrino flux determined from non-detection of deeply penetrating particles by the Fly's Eye detector.

upper limit on the monopole abundance – the so-called ‘Parker limit’ (see ref. 22) – and is, therefore, quite plausible.

From Figure 3 we see that TD models are probably not relevant for cosmic rays below about 5×10^{19} eV; it is only at energies above this energy that TD models become a viable option. It is to be mentioned here that the shape of the proton spectra in the acceleration models (not shown in Figure 3), which typically yield power-law injection spectra with index $\alpha \geq 2$, would correspond to a sharply falling curve at energies beyond $\sim 10^{20}$ eV, and so would be unable to explain the two highest energy events indicated in the figure. The ‘dip’ of the proton curve of Figure 3 beginning at $\sim 10^{20}$ eV and its subsequent ‘recovery’ at $\sim 10^{21}$ eV are characteristic features induced by the propagation effects (the GZK effect discussed in §3). The ‘recovery’ after the ‘GZK cut-off’ of the proton spectrum in Figure 3 is, however, a feature that is not shared by the proton spectra one gets in standard acceleration models, which as mentioned above, do not ‘recover’ after the GZK cut-off. (For detailed understanding of this point see, e.g. figure 1 of ref. 17.) The recovery in the present case is essentially due to flatter nature of the proton injection spectrum in the TD model compared to that in the acceleration models. Note also from Figure 3 that the two highest-energy events are naturally explained in the TD model only if these are γ -ray events, and not protons. Experimentally, it is hard to determine the composition of the events at these energies with full certainty. Although, the traditionally favoured composition for these events are protons, a γ -ray composition is certainly not ruled out⁸.

Figure 3 also indicates a peculiar feature of the present HECR data: There is an apparent ‘gap’ in the spectrum; the two highest-energy events are separated from the rest of the data by almost half a decade in energy. Possible implications of this apparent ‘gap’ in the spectrum for theories of origin of HECR have recently been analysed¹⁸ where it is concluded that although an acceleration origin of the HECR cannot be entirely ruled out with the current data statistics, the persistence of the apparent gap in the existing data at a quadrupled total exposure of the detector (as might be expected to be achieved within the coming few years) would rule out many acceleration models at more than 99% confidence level. In that case, one may have to take recourse to some kind of non-acceleration scenario for the origin of HECR like the TD model described above.

Besides the characteristic shapes of the HECR spectra, the TD models of HECR origin have two definitive predictions. Firstly, the HECR should consist of only ‘fundamental’ particles like protons, neutrons, γ -rays, neutrinos, electrons, positrons, and so on (and perhaps their antiparticles too), but definitely *no nuclei* such as ${}^4\text{He}$ or Fe. (There is no way hadronization of quarks

would directly give rise to nuclei!) Secondly, the HECR should be highly rich in γ -rays and neutrinos. These predictions can be used as crucial tests of the TD model in future HECR experiments with large-area detector coverage, such as the proposed Pierre Auger project²³. More details on the subject of topological defect scenario of origin of HECR can be found in refs 24, 25.

5. Summary and conclusions

We have discussed rather sketchily the present status of the subject of cosmic rays, the origin of which is one of the major unsolved problems in physics and astrophysics. There are reasons to believe that cosmic rays with energy up to the ‘knee’ region of the spectrum corresponding to the energy $\sim 10^{15}$ eV are produced by some kind of acceleration process operating in the relativistic shocks associated with supernovae, and those from about 10^{15} eV up to the ‘ankle’ region at $\sim 5 \times 10^{18}$ eV are produced by acceleration processes in pulsar magnetospheres. The sources of these components are thought to lie within our Milky Way Galaxy, although the highly tangled nature of the trajectories of these particles (caused by the non-uniform galactic magnetic field) prevents direct identification of these sources with the known supernova remnants and pulsars. The component beyond $\sim 5 \times 10^{18}$ eV is thought to be of extragalactic origin. As far as the highest-energy cosmic rays (i.e. those with energy above $\sim 10^{20}$ eV) are concerned, the known acceleration processes are found to be inadequate from the point of view of energetics. We have discussed a possible alternative *non-acceleration* scenario for the origin of this HECR component. This scenario involves new physics; in particular, it involves formation and destruction of cosmic topological defects in the form of cosmic strings, monopoles, etc. that are predicted in theories of early universe that bring together modern ideas of high-energy particle physics and the big-bang model of cosmology. The highest-energy end of the cosmic ray spectrum thus offers a probing ground for testing some of these new ideas.

We have skipped many issues that have important bearings on the question of origin of cosmic rays in general, such as the issue of isotropy of the observed cosmic rays, the possible important roles of γ -ray astronomy as well as neutrino astronomy in unravelling the mystery of cosmic rays, and so on. It is hoped, however, that the reader will get a general flavour of the outstanding open issues in modern cosmic ray physics, issues which undoubtedly constitute one of the most exciting areas of current research in contemporary physics, astronomy and astrophysics.

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Is the Hubble flow a result of inverse cascade?*

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A few general characteristics of nonlinear open systems are described. A turbulent fluid is one such system which exhibits order by supporting structures in an otherwise random medium through the transfer of energy from small spatial scales to large spatial scales. The spatial distribution of energy so derived is found to account well for two disparate situations like the solar granulation and the rotation curves of galaxies. Encouraged by these successes, one wonders if the spatial distribution of energy at the largest scales, i.e. $V(L) \propto L$ has anything to do with the Hubble flow.

COHERENT structures, correlated motions and well-defined patterns are observed on a variety of spatial as well as temporal scales. Organized states of matter and motion can be seen in a convection cell, cloud complexes, a tornado, a cyclone, zonal flows on planetary surfaces, the Red Spot of Jupiter, convective flows on stellar surfaces, spiral patterns in galaxies, clusters of galaxies and perhaps ourselves. Figure 1 a-c represents distribution of clouds in the earth's atmosphere, of convective

motions on the solar surface and of galaxies in clusters of galaxies. Could you tell one from the other? Figure 2 a, b represents the velocity vectors in a cluster of galaxies and on the solar photosphere. Both show converging and diverging flows. The usual interpretation for clusters of galaxies is infall of matter in the strong gravitational field of the unseen dark matter, whereas the solar photosphere acquires the same pattern due to the formation of fluid vortices. Can the vortices account for the flow patterns in clusters of galaxies? Is the invisible matter indispensable? In other words, do these organized states of matter and motion arise under equilibrium or non-equilibrium conditions? Is it substance and or style? Are these dissipative structures?

Our proposal¹⁻⁶ is that apparently disparate phenomena of (i) non-equilibrium motions on stellar surfaces, (ii) the large scale organization of matter, motion and magnetic field or in general the large scale structure of the universe have their origin in the inverse cascade of energy leading to self-organization in an otherwise nonlinear turbulent medium.

Novelties of non-equilibrium systems

Near equilibrium, a system, when perturbed, comes back

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to its initial state and is said to be stable. Under far-from equilibrium conditions, a system, when driven sufficiently far, may never return to its initial state and is said to be unstable. The system undergoes a bifurcation, where the initial solution becomes unstable and the new state behaves in a manner completely different from that of the initial state. The new state that emerges after a bifurcation is known as a dissipative structure. A spectacular example of this phenomenon is provided by

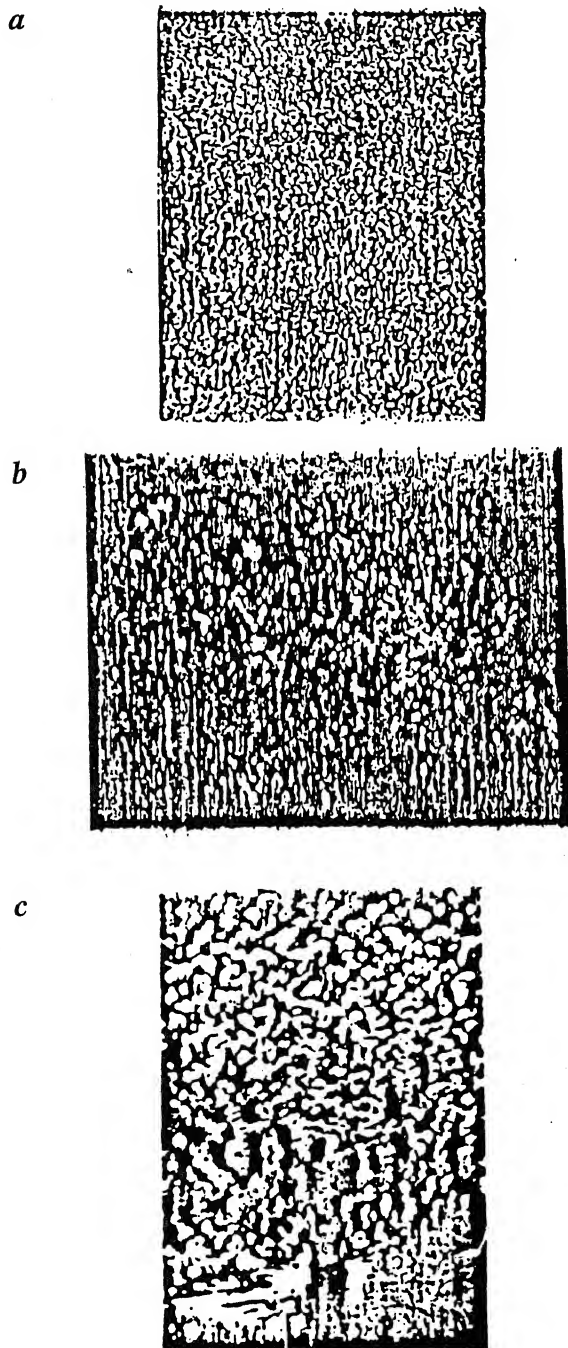


Figure 1a-c. Distribution of clouds in the earth's atmosphere of convective motions on the solar surface and of galaxies in clusters of galaxies.

chemical clocks. A bifurcation is associated with the breaking of a symmetry. One starts with a system describable by completely symmetric equations and finds their solutions which are not symmetric, i.e. a bifurcation splits into a so-called right handed and a left handed solution. The physical reality may be embedded in only one of these solutions. For example, sea shells usually have a preferential chirality. It is seen that in a highly unstable dynamical system, the trajectories starting from infinitesimally close points diverge exponentially in time and such a system is said to be in a chaotic state. Thus a completely deterministic description supports a chaotic solution, which lends it a probabilistic behaviour, i.e. the system is intrinsically random and irreversible. For example, a collection of particles moving parallel to each other, develops randomness with the passage of time, i.e. they try to reach equilibrium. Now time inversion symmetry requires that the reverse must happen, i.e. randomly directed velocity vectors must align themselves with the passage of time. But this is never observed (Figure 3). This means that we need representations of dynamics which are not invariant with respect to time inversion and such representations do exist for highly unstable systems. This reduces the predictability of a system. Tomorrow is not already present in today! Instead, it emerges in an unpredictable way, the way improvization is achieved in Indian music.

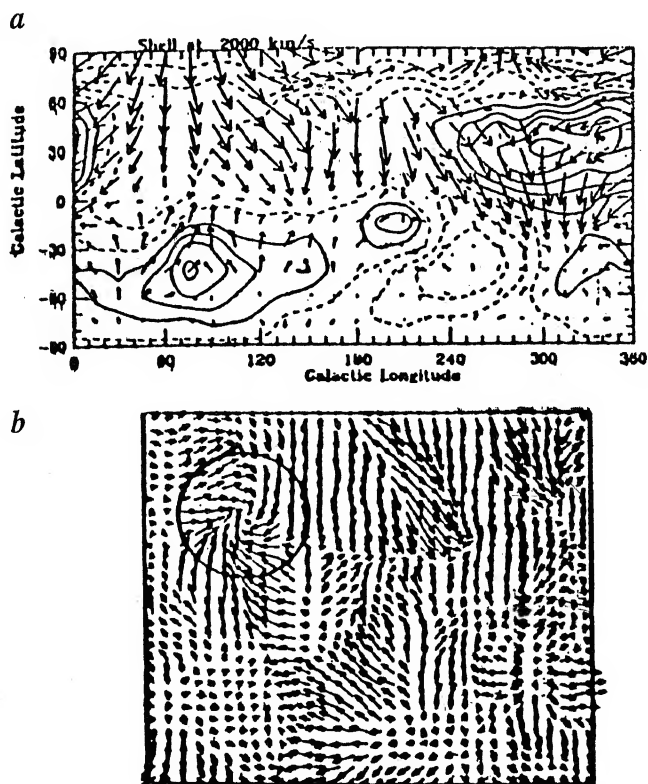


Figure 2. Velocity vectors in a cluster of galaxies and on the solar surface.

It is in intrinsically random, irreversible, unsymmetric, non-equilibrium systems that all phenomena of material existence are embedded. The conditions for formation of dissipative structures can therefore be summarized as^{7,8}: (i) a macroscopic system far from equilibrium, so that it can develop correlations among its different parts, (ii) the system is open towards its environment, i.e. it can draw from its surroundings, and (iii) it should be able to self-reinforce itself autocatalytically by a sustained level of fluctuations deriving from its neighbourhood.

Fluid turbulence

Far from equilibrium conditions exist everywhere in the universe, including its origin, the big bang. Many structures like galaxies and clusters of galaxies are suggested to form and exist in non-equilibrium states. The belief in equilibrium has led us to darkness, since, equilibrium commands that most of the matter in the universe remain invisible. To investigate the role of non-equilibrium nonlinear processes in astrophysical objects, we need to go to the fluid description since several aspects of stellar, galactic and the universe as a whole can be understood by treating them as fluids, always in a state of turbulence. The fluid turbulence is a tricky subject. Even Heisenberg found the problem too difficult. He is reputed to have said that he hoped, before he dies, some one would explain quantum mechanics to him, but after he died, he hoped, God would explain turbulence to him⁹. Nevertheless, a few characteristics of fluid turbulence can be summarized here. It is a random state of fluid motion that supports several interacting length and time scales through the excitation of instabilities. The scales are constrained by boundaries, buoyancy and dissipation. Turbulence affects all transport and diffusion

processes, generally leading to an enhanced efficiency.

Quantification of turbulence

Mean values of the products of field variables (like velocity U , magnetic field, etc.) and their derivatives form the fabric of a turbulent medium. Out of these, the two-point correlation function $R_{ij}(\mathbf{r})$ defined as

$$R_{ij}(\mathbf{r}) = \langle U_i(\mathbf{X}) U_j(\mathbf{X} + \mathbf{r}) \rangle \quad (1)$$

is the most important. Here, the angular brackets represent the space average. For homogeneous and stationary turbulence $R_{ij}(\mathbf{r})$ depends only on the configuration and not on its location. The Fourier transform $\phi_{ij}(\mathbf{K})$ of $R_{ij}(\mathbf{r})$ is defined as:

$$\phi_{ij}(\mathbf{K}) = \frac{1}{(2\pi)^3} \int R_{ij}(\mathbf{r}) e^{-i\mathbf{K}\cdot\mathbf{r}} d^3r. \quad (2)$$

The total kinetic energy W per unit mass of the fluid is given by

$$W = \frac{1}{2} \langle U_i(\mathbf{X}) U_i(\mathbf{X}) \rangle = \frac{1}{2} \int \phi_{ii}(\mathbf{K}) d^3K = \int E(K) dK, \quad (3)$$

where $E(K)$ is the omnidirectional energy spectrum. From the Kolmogorov law, that in a quasi-steady state, there should be a stationary flow of energy in K space from the source to the sink, i.e. the energy density flow rate should be a constant and equal to the dissipation rate E of the energy density at the sink, one finds the energy spectrum

$$E(K) \sim \varepsilon^{2/3} K^{-5/3} \quad (4)$$

describing the flow of energy from large spatial scales to small spatial scales. But what if the assumptions of homogeneity and isotropy are dropped?

Inverse cascade in 2D turbulence

In a 2D incompressible and ideal system, there are two invariants, the total energy W and the enstrophy U_E defined as:

$$U_E = \int \frac{(\nabla \times \mathbf{U})^2}{2} d^3r. \quad (5)$$

Therefore one derives two types of inertial ranges, one for the energy and the other for the enstrophy. The two energy spectra are:

$$E(K) \sim \varepsilon^{2/3} K^{-3} \quad \text{for enstrophy} \quad (6)$$

and $E(K) \sim \varepsilon^{2/3} K^{-5/3}$ for energy and it has been argued

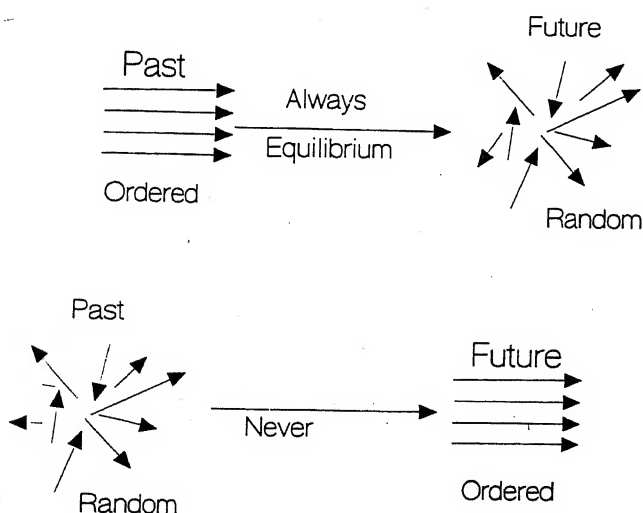


Figure 3. Irreversible processes.

and demonstrated that energy cascades from small spatial scales to large spatial scales according to $K^{-5/3}$ law, thus setting up an inverse cascade¹⁰.

Inverse cascade in 3D turbulence

The appearance of large scale structures in the 3D atmospheres of planets compels us to look for the possibility of inverse cascade in 3D systems. Specifically, the observations of helical type of flow structures in circumstances varying from oceans to cloud complexes, brought to the fore, the importance of helicity in turbulent fluids. The fundamental idea that needed to be appreciated was that the large helicity fluctuations always exist in a turbulent medium, even if the average helicity vanishes. It was shown that the fluctuating topology of the vorticity field in turbulent flows can be characterized by the statistical helicity invariant I represented by conserved mean square helicity¹¹⁻¹³:

$$I = \int \langle h(\mathbf{X}) h(\mathbf{X} + \mathbf{r}) \rangle d^3r, \quad (7)$$

where

$$h = \mathbf{U} \cdot \nabla \times \mathbf{U}.$$

Thus E and I are the two invariants of a helically turbulent 3D system. Again using Kolmogorovic arguments for the I invariant, one finds the spectrum in the inertial range as

$$E(K) \propto K^{-1}$$

and the total energy density

$$W = \int E(K) dK \propto \log [L(t)/l]. \quad (8)$$

Here $L(t)$ is the transient length scale excited at time t . One observes that the energy grows very slowly as the spatial scale $L(t)$ grows. So, practically, there is little transfer of energy towards large scales. But, what happens is that as the correlation length of helicity fluctuations increases, the velocity and vorticity become more and more aligned and as a consequence the non-linear term $(\mathbf{V} \cdot \nabla)\mathbf{V}$ of the Navier-Stokes equation decreases and flow of energy towards small scales is retarded. On the other hand, the growth of correlation length cannot go on indefinitely. Especially if the medium is restricted in the vertical direction by gravity or buoyancy as is true of atmospheres of any celestial object, may it be a planet or a star. Under such circumstances, the correlation length continues to grow in the horizontal plane and the system becomes more and more anisotropic. In addition, $I(K)$ is found to be

dominant at small K while $E(K)$ is larger at large K and the inverse cascade of I follows.

What is achieved by the growth of correlation length of helicity fluctuations is the anisotropy in the system which can now be approximated to a quasi 2D system. Here, the horizontal scale is much larger than the vertical scale and the vertical velocity is smaller than the horizontal velocity. Under these conditions, the energy and the I spectrum become identical and go as $K^{-5/3}$, as in the 2D case. One expects that an increasing fraction of energy is transferred to large spatial scales as the anisotropy in the system increases. This can go on until coriolis force begins to be effective. The length scale L_c where the nonlinear term of the Navier-Stokes equation becomes comparable to the coriolis force is given by $L_c = U/\Omega$, where Ω is the angular velocity. At these large scales, the system simulates 2D behaviour and enstrophy conservation begins to play its role. One may consider scales $L \geq L_c$ as a source of vorticity injection into the system. The enstrophy then cascades towards small scales with a power law spectrum given by:

$$E(K) \propto K^{-3}$$

$$W \propto L^2. \quad (9)$$

Thus the complete energy spectrum of a hydrodynamic turbulent medium is given in Figure 4. What is the evidence for this spectrum?

Solar granulation

The cellular velocity patterns observed on the solar surface are believed to be manifestations of convective phenomena occurring in the sub-photospheric layers. The quality observations obtained at Pic-du-Midi indicate the existence of a continuum of sizes instead of one or two

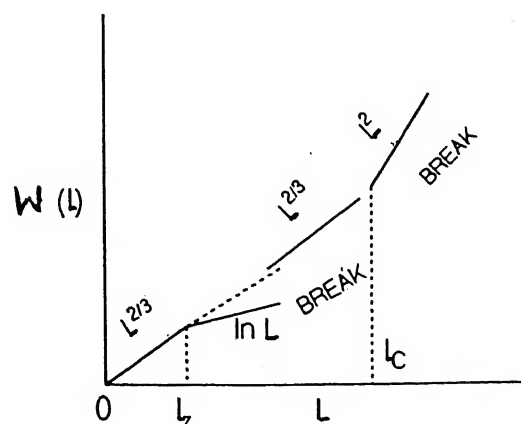


Figure 4. Turbulent energy spectrum, L_z , normalizing length; L_c , break due to coriolis force (Krishan^{1,2}).

dominant scales in the solar granulation. We have proposed an inverse cascade model for the entire granulation phenomenon. It is encouraging to find that the energy spectrum of granular motions deduced from observations does show a branch with $K^{-5/3}$ which turns into a $K^{-0.7}$ law towards small K (Figure 5). This agrees fairly well with the predictions of the inverse cascade model where the Kolmogorov branch $K^{-5/3}$ develops into a K^{-1} behaviour towards large spatial scales. Thus the solar granulation provides one example where the flat branch K^{-1} seems to account well for an observed phenomenon. Is there another example?

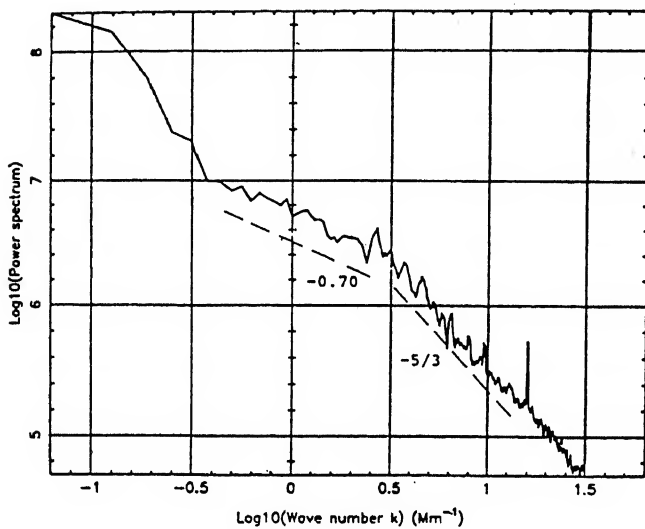


Figure 5. Power spectrum of the solar photospheric motions (Zahn¹⁵).

Rotation curves of galaxies

The issue of the flat rotation curves of galaxies and the need for dark matter is described precisely in Figure 6. The flat nature of the orbital motion in galaxies is accounted through the relationship $\frac{1}{2} mV^2 = (GMm/R)$ by assuming that $M \propto R$ and therefore, the velocity $V = \text{constant}$. Since the mass $M \propto R$ has no luminosity associated with it, it is known as dark matter. We present an alternative explanation for the flat nature of the velocity by applying the ideas of inverse cascade in a turbulent atmosphere of a galaxy. We propose a law of velocities of the following type:

$$V(L) = AL + BL^{1/3}$$

in the inner region, i.e. for $L < L_z$ and (10)

$$V(L) = CL^{-1/2} + D[\ln L/L_z]^{1/2}$$

in the outer region, i.e. for $L > L_z$ of a galaxy. A, B, C and D are determined from the fits with the observed velocity curves. We have successfully accounted for the flat nature of the rotation curves for nearly hundred galaxies. An example is given in Figure 7.

We have discussed two cases, from completely disparate phenomena, where the Kolmogorov branch $K^{-5/3}$ and the flat branch K^{-1} together have explained the observed behaviour very well. What about the rest of the spectrum given in Figure 4? Particularly, is there any evidence for the spectrum at the largest spatial scales, i.e. for $E(K) \propto K^{-3}$ or $W(L) \propto L^2$?

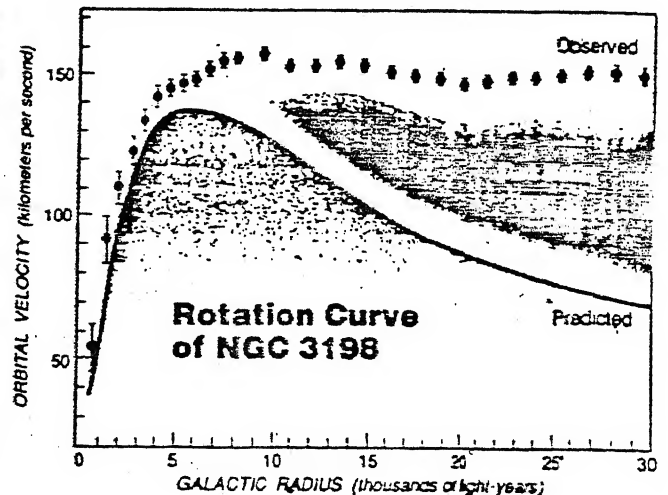
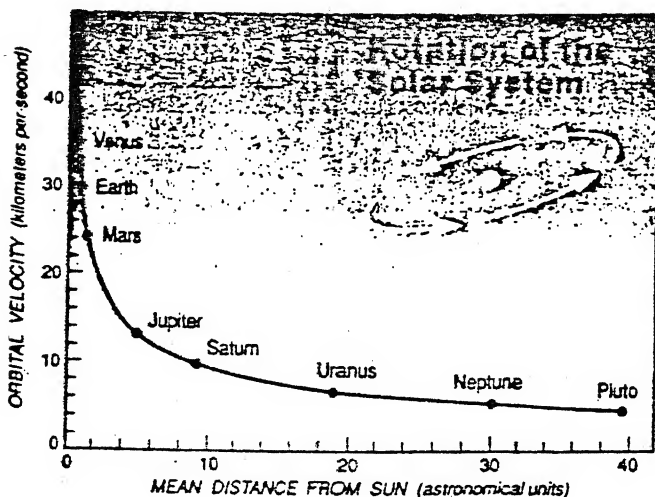


Figure 6. The case for dark matter in spiral galaxies. *Left*: The orbital velocities of the planets (dots) decrease with distance from the Sun exactly as predicted by Newtonian gravitation (line), assuming a system dominated by one solar mass at its center. *Right*: The cosmos is not as well behaved on galactic scales. Here a graph of orbital velocity versus radius has been computed for NGC 3198, a spiral galaxy in Ursa Major, assuming that the distribution of light serves as a good indicator of the distribution of mass. The failure of the observed velocities (dots) to match the predicted ones is striking and points to an unseen component of dark matter in the galaxy.

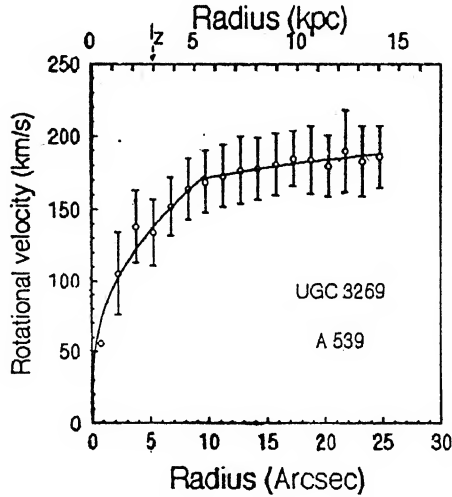


Figure 7. Flat rotation curve of a galaxy fitted by equation (10).

The Hubble flow

The energy spectrum at the largest spatial scales is governed by enstrophy transfer and goes as

$$E(K) \propto K^{-3}. \quad (11)$$

Putting in the proportionality constants, the total energy density per unit gram varies as

$$W(L) = V^2(L) \simeq (\varepsilon/L_c^2)^{2/3} L^2$$

or

$$V(L) \simeq (\varepsilon/L_c^2)^{1/3} L. \quad (12)$$

$\varepsilon = V^2/\tau$ is the energy injection rate and (ε/L_c^2) is the enstrophy injection rate. Substituting for L_c , the scale at which the coriolis force becomes comparable to nonlinear inertial force, one finds

$$V(L) \simeq (\Omega^2/\tau)^{1/3} L \quad (13)$$

which, when compared with the Hubble flow expressed as

$$V = HL$$

gives the value of the Hubble constant H to be

$$H = (\Omega^2/\tau)^{1/3}. \quad (14)$$

Does this mean that the velocities with which the galaxies are receding from each other like dots on an expanding balloon are given by the largest spatial scale end of the same energy spectrum whose small spatial scale part accounts so very well for the flat rotation curves? If one assumes that the angular speed at scales $\sim L_c$ is such that $\Omega^{-1} = \tau$, then one gets $H^{-1} = \text{age of the universe} = \tau$, the duration for the injection of energy. If stars are the main contributors to turbulence, the age of the universe τ is nothing but the age of the oldest stars. Of course, the big bang itself is a source of turbulence in the universe.

Conclusion

The need of the hour is to think non-equilibrium. This may alleviate some of the caveats that arise from the belief that equilibrium rules the universe. Hydrodynamics in addition to gravitational processes must be included in the scheme of things since a self-consistent distribution of matter and motion must be determined. One must realize that converging flows commonly occur in a turbulent fluid without the necessity of invoking great masses. The whole question of mass distribution or the large scale structure of the universe can only be settled by including the physics of hydrodynamical processes in the making of structures. The angular momentum must be treated with reverence!

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New measurements of the Hubble constant and its implications*

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One of the key projects on the Hubble Space Telescope (HST) is to determine the distance to the Virgo cluster of galaxies using classical Cepheid variables as standard candles. The refurbished HST has resolved and measured the periods and magnitudes of twenty odd Cepheids in M100, a spiral galaxy near the core of the Virgo cluster. Coincidentally, ground-based observations from Hawaii with the help of a high resolution camera, under subarcsecond seeing conditions, have also succeeded in the identification of a few Cepheids in NGC 4571, another Virgo spiral. The distance to the Virgo cluster from these two sets of measurements turns out to be 17.1 ± 1.8 Mpc and 14.9 ± 1.2 Mpc respectively and implies a value of the Hubble Constant H_0 equal to 80 ± 17 km s⁻¹ Mpc⁻¹ and 87 ± 7 km s⁻¹ Mpc⁻¹. The result implies that the age of the Universe is in the neighbourhood of 10 ± 2 Gyr. Some of the globular clusters in the Milky Way system are known to be older (age ~ 14–18 Gyr) from stellar evolutionary calculations. Secondly, another important standard candle, the Type Ia supernova, has now been accurately calibrated using HST data on Cepheids in IC 4182 and NGC 5253, the host galaxies for SN 1937C and SN 1972E respectively. These calibrations yield a much lower value of H_0 from the Hubble diagram of SNe Ia.

Though there may still be uncertainties of the order of 10–20% in the measurements, the low value of H_0 from SN Ia measurements focuses attention on the class of objects classified as SN Ia, and their suitability as standard candles. Significantly, the high value of H_0 from the measurements of classical Cepheids may indicate that we live in a Universe somewhat different from the one we have been thinking we do.

Ever since Hubble's discovery of the universal recession of galaxies as a result of the cosmological expansion, measurement of the rate of this expansion, that is its current value has been a subject of intense investigation. The Hubble parameter H_0 , referred to as the Hubble Constant is related to the factor $R(t)$ which determines the rate of the isotropic expansion of a homogeneous Universe through the relation

$$H_0 = \dot{R}/R. \quad (1)$$

H_0 is an observationally determinable parameter as it

*Based on a talk presented at the Symposium on 'Interface of Astronomy with other Sciences', organized by Indian National Science Academy and held at the Indian Institute of Astrophysics Observatory, Kodaikanal, on 4–5 May 1995.

appears in the velocity–distance relation of receding galaxies given by

$$V_r = H_0 \times D. \quad (2)$$

Here V_r is the velocity of recession usually measured as a redshift of the spectral lines from distant galaxies and D is the distance to the galaxies. As velocities are measured in km s⁻¹ and the distances in megaparsecs (1 parsec = 3.085×10^{13} km) H_0 is usually expressed in the units of km s⁻¹ Mpc⁻¹ and has the dimension of inverse time. The reciprocal of H_0 is the age of the Universe. In the present century, measurement of H_0 has been one of the most important goals of observational astronomy.

To derive H_0 two measurements are needed, the velocity of recession of a faraway galaxy and its distance. Though the velocities are estimated quite accurately from the redshift of the spectral lines, distances to astronomical objects are difficult to determine. And it is the uncertainty in the distance estimates that has hindered an accurate determination of H_0 since the 1930s. Hubble's original estimate of 500 km s⁻¹ Mpc⁻¹ for H_0 (ref. 1) was revised to about 250 km s⁻¹ Mpc⁻¹ by Baade² when he recognized the population differences between classical Cepheid and RR Lyrae variables and revised the luminosities of the former upward by 1^m.5. A further revision to $H_0 = 75$ km s⁻¹ Mpc⁻¹ was effected by Sandage³ when he discovered that on the photographic plates objects, thought earlier to be the brightest stars in distant galaxies were, in fact, small clusters of stars or compact knots of ionized hydrogen regions around these clusters. Since the 1960s the estimated values of H_0 have ranged between 50 km s⁻¹ Mpc⁻¹ and 100 km s⁻¹ Mpc⁻¹, creating an uncomfortably large range of a factor of two in the inferred size and age of the Universe. A curious feature of these estimates is that the value of H_0 shows a bimodal distribution with peaks at approximately 50 and 90 km s⁻¹ Mpc⁻¹ which is directly related to the two widely different values of the distance to the Virgo cluster obtained by the use of methods falling in two mutually exclusive categories. The 'short' distance to Virgo ($D \sim 15$ Mpc) leads to the larger value of H_0 while the 'long' distance to Virgo leads to an H_0 in the neighbourhood of 50 km s⁻¹ Mpc⁻¹. Each is supported by measurements involving secondary distance calibrators. Although almost all these methods yield consistent results in the Local Group and beyond, they seem to suddenly

fall out with one another at the nearest cosmologically significant distance, that is the distance to the Virgo cluster.

Galaxies are known to cluster on various scales. The Local Group of twenty odd galaxies, which the Milky Way is a member of, is part of a larger group, the Local Supercluster containing within it several groups and clusters including the Virgo cluster. Within the group, galaxies evince a complex pattern of motion that has little to do with the uniform Hubble flow. Thus to obtain an estimate of H_0 astronomers have to look at clusters of galaxies at distances large enough that the random motions of the galaxies within the group are a tiny fraction of their recessional motion as part of the uniform Hubble flow. Since direct trigonometric methods do not carry us far enough even within the Milky Way, astronomers have always relied heavily on indirect methods to estimate distances to faraway objects. Using direct trigonometric methods to obtain distances and hence the luminosities of bright stars, they have extended distance estimates to remote regions of the Galaxy and nearby galaxies using the bright stars as the standard candles. At distances where the bright stars are too faint to be seen, secondary standard candles like globular clusters, SN Ia, HII regions, etc. have been devised and used to estimate distances. Farther out a tertiary standard candle like the brightest galaxy, or the largest galaxy, in a cluster has been used and eventually large enough distances are reached where the unperturbed uniform expansion can be clearly discerned. A velocity estimate at this distance obtained from the redshift of the spectral lines of the test object then leads to H_0 . This stepwise process, wherein new distance indicators are calibrated using indicators from the previous step, is liable to accrue both random and systematic errors lending a large uncertainty in the value of H_0 that is eventually estimated. Elimination of a step in the process could improve the accuracy of the distance determination considerably and ways and means of effecting this have occupied the attention of astronomers.

Direct measurement of magnitudes and periods of classical Cepheid variables in the Virgo cluster was long recognized to be a potentially powerful way that leapt over a crucial step in the cosmological distance ladder by eliminating the need of secondary calibrators to obtain the distance to this cluster. The use of Cepheids involves only one step – the calibration of their period–luminosity relation by direct trigonometric means, known since the days of Harlow Shapley. Since the Earth's atmosphere hampers attempts at resolving Cepheid variables at the distance of the Virgo cluster, the need for observations from a satellite-based telescope was recognized. The Hubble Space Telescope (HST) has now performed this task by resolving a large number of classical Cepheids in the spiral galaxy M100, a member

of the Virgo cluster. Near-simultaneous advances in optical and detector technologies have enabled astronomers to beat the 'seeing' to a large extent and using a high resolution camera at the Canada–France–Hawaii Telescope (CFHT) atop Mauna Kea, Hawaii, a group has been able to resolve a handful of the brightest classical Cepheids in NGC 4571, another member spiral of the Virgo cluster. The distance estimates to both the spirals, whose positions in the sky are close to the assumed dynamical centre of the cluster, support the long distance scale, implying a value of H_0 close to $80 \text{ km s}^{-1} \text{ Mpc}^{-1}$. This direct distance estimate to the Virgo cluster suggests a Universe no older than 12 Gyr.

Such a young Universe is bothersome since stellar astronomers have gathered ample evidence of stars and clusters of stars in our Galaxy which appear to be older. Are all stellar age estimates then wrong? A second equally important problem has been thrown up by measurements from the very same HST, this time of supernovae of Type Ia. While these supernovae are only secondary distance indicators, their luminosities have now been directly calibrated by using classical Cepheid variables in the galaxies NGC 5253 and IC 4182 where the prototype SNe Ia 1972E and 1937C were observed. Using SN Ia as a standard candle one obtains quite a different value of H_0 , namely $H_0 = 52 \text{ km s}^{-1} \text{ Mpc}^{-1}$ which is in full agreement with the 'long' distance scale and hence an older Universe. Finally, a value of H_0 in the neighbourhood of $80 \text{ km s}^{-1} \text{ Mpc}^{-1}$ will rule out any preferred cosmological model with the density parameter $\Omega_0 = 1$ unless the cosmological constant is nonzero.

Classical Cepheids as primary distance indicators in an expanding Universe

Although classical Cepheid variables had been known since the late eighteenth century, their prime importance as distance indicators was first realized in the 1910s when Henrietta Leavitt published the results of her study of these stars in the Small Magellanic Cloud (SMC). Leavitt noticed that in the SMC the Cepheid variables with longer periods had higher average apparent brightness. Since all stars in the SMC were logically at about the same distance from us, it was immediately obvious that Leavitt's correlation implied the existence of a period–luminosity (P–L) relation for these variables. The significance of this discovery was realized soon by Harlow Shapley then working at Mt. Wilson, California. Shapley used the method of statistical parallaxes on a handful of Galactic Cepheids, for which proper motion data were available, to obtain a calibrated P–L relation. Shapley had noticed that the globular clusters invariably contained short period variables called the RR Lyrae variables whose light curves were no different from those of the classical Cepheids. He extended the P–L

relation to include the RR Lyrae variables obtaining thereby their intrinsic luminosities. His next step was to use these variables to obtain the distances to globular clusters and then to map their distribution as part of our galactic system. The sequence of discoveries by Shapley which finally gave a reasonable idea of the size and shape of our Galaxy and the position of the solar system in it has often been likened to the Copernican revolution which ushered in the scientific renaissance in the 1600s.

In the 1920s when the nature of the spiral nebulae was still hotly debated upon, Edwin Hubble using the newly commissioned 100" telescope at Mt. Wilson was able to resolve Cepheids in the Andromeda nebula and provided the first measurement of a distance beyond our Galaxy. The existence of external galaxies was proved beyond doubt. Armed with the powerful tool of Cepheid variables as standard candles, Hubble and then others extended distance measurements to the Local Group galaxies and beyond. Using Cepheids in the nearby regions and then devising secondary indicators (e.g. brightest stars, supernovae, globular clusters, etc.) which were calibrated with respect to the Cepheids and RR Lyr variables to go further, and finally by using these secondary indicators to calibrate galaxy diameters and galaxy magnitudes in clusters, astronomers, during the decades following Hubble's work were thus able to reach out to cosmological distances. The series of efforts resulted in the refinement and extension of the calibrated velocity distance relation that was first given by Hubble in 1929.

Cepheid variables are thus seen to have played a seminal role in setting up the extragalactic distance scale by being the first step of the cosmological distance ladder.

Cepheids are a class of regular pulsating variable stars with periods ranging from a couple of days to well over a hundred days. They are of spectral types F to G and are intrinsically bright, belonging to the luminosity classes of bright giants and supergiants. Their internal structure and evolutionary status as core-He burning stars are well understood and highly sophisticated models are available that reproduce all of their observed properties. On the HR diagram they occur in a narrow strip somewhat inclined to the temperature/colour axis and it was observationally shown that their period depends both on the luminosity and the colour. The P-L-C relation, which is of fundamental importance in measuring extragalactic distances, has also been derived theoretically from the models and it is in excellent agreement with the P-L-C relation determined empirically. The best calibration of the relation comes from Cepheids in open clusters whose distances are known to great accuracy through the fitting of their main sequences to the Hyades main sequence, the latter being calibrated directly in absolute magnitudes through the method of moving cluster parallaxes. Thus the study of Cepheids is very

secure both theoretically and observationally and hardly any substantial change will occur in any of the results based on the properties of these stars.

Distances to the Local Group galaxies and to other galaxies slightly beyond have been estimated using Cepheid variables as standard candles. A variety of other calibrators have also been used to estimate the distances to these galaxies. The best measurements to date are available for the Magellanic Clouds. In their case the distance moduli obtained by different methods agree to within $0''.10$. Until the HST and the CFHT measurements the farthest the Cepheids were seen were in M101 at a true distance modulus $(m - M)_0 \sim 29''.5$. Even at this distance the other methods led to distance estimates in good agreement with the ones obtained through Cepheids. However, the distance is yet too small to see an unambiguous Hubble flow. Determination of cosmologically significant distances for deriving H_0 had depended totally on the use of secondary distance calibrators.

Distance estimates to the Virgo cluster

The Virgo cluster forms the core of the Local Super-cluster. Its role in the determination of the extragalactic distance scale has been crucial. The cluster is large and it contains the full complement of galaxies of different morphological types allowing for the application of almost all the methods of distance estimates at hand including those using novae and supernovae of different types since the richness of the cluster ensures that even these rather rare events are not too infrequent in it. Further the cluster is at a high galactic latitude such that the foreground extinction to it is not a problem. It is ideally located for observations from both hemispheres of the globe. And lastly it is sufficiently distant so that most of its recessional velocity derives from the Hubble expansion.

Before 1994 the distance estimates to this cluster were all based on secondary and tertiary calibrators since technology did not allow yet the discovery of Cepheids in its member spirals. Table 1 lists these estimates from a variety of sources. Dichotomy of the results is quite evident. While surface brightness fluctuations, the planetary nebula luminosity function and the Tully-Fisher relation lead to a short distance to the cluster

Table 1. Distance of the Virgo cluster

Method	Author and ref.	$(m - M)_0$
Planetary nebulae	Jacoby <i>et al.</i> ¹²	30.74 ± 0.05
Surface brightness fluctuations	Tonry <i>et al.</i> ¹³	30.86 ± 0.13
Tully-Fisher	Pierce and Tully ¹⁴	30.88 ± 0.22
Globular clusters	Harris <i>et al.</i> ¹⁵	31.47 ± 0.25
Novae	Capaccioli <i>et al.</i> ¹⁶	31.30 ± 0.36
SN 1979C in M 100	Schmidt <i>et al.</i> ¹⁷	31.40 ± 0.60

$(m-M)_0 \sim 30.7$, SN Ia, the globular cluster luminosity function and novae lead to a long distance with $(m-M)_0 \sim 31.4$. The long distance is also supported by measurements that use diameters or scale lengths of Sc I galaxies as calibrators. The difference in the distance moduli between the two sets, which is nearly a full magnitude, translates to a distance ratio of about 1.4. This large difference has been principally responsible for the controversy surrounding the value of the Hubble parameter H_0 , since the dispersion in the velocity estimates of the member galaxies of the Virgo cluster produces a much smaller uncertainty. It has also been noted in the literature^{4,5} that though the absolute distance to the Virgo cluster was in dispute, the relative distances of distant clusters with respect to Virgo were not. A variety of methods yielded very similar relative distances to the Coma cluster with uncertainties in $D(\text{Coma})/D(\text{Virgo})$ which are less than 10%, more typically on the order of 5%. It was thus realized that an accurate distance estimate to the Virgo cluster using a primary calibrator could lead to an equally accurate absolute distance to the Coma cluster where the Hubble recessional velocity is easily an order of magnitude larger than the typical random motions of galaxies within the cluster. This would then lead to an accurate determination of H_0 . One of the key projects assigned to the Hubble Space Telescope was thus the discovery and measurement of classical Cepheid variables in the spirals of the Virgo cluster. It is my personal belief that the eventual naming of the Space Telescope was consciously or unconsciously influenced by this very important task that it was to undertake.

HST and CFHT observations of Cepheid variables in Virgo cluster

In 1994 two independent groups^{4,6} announced the first discovery of classical Cepheid variables in the spiral galaxies NGC 4571 and NGC 4321 (M 100) which are members of the Virgo cluster. While twenty odd Cepheids were resolved by the Hubble Space Telescope in M 100, ground-based efforts using a sophisticated high resolution camera (HR Cam) on the Canada-France-Hawaii Telescope atop Mauna Kea, could discover three Cepheids in NGC 4571.

Cepheids in M 100

M 100 is a giant Sc galaxy with a projected location $3^\circ.9$ away from the centre of the cluster defined by the giant elliptical galaxy M 87 (Virgo A, NGC 4486). The HST measurements have covered full cycles of about twenty Cepheids in this galaxy with periods ranging from 20 to 65 days. Both V band and I band measurements were made. The mean V and I magnitudes

and the measured periods define a nearly perfect linear relation that was indistinguishable in its slope from the absolute P-L relation of the LMC Cepheid sample. The reddening-corrected true distance modulus led to $D(\text{M 100}) = 17.1 \pm 1.8$ Mpc based on an LMC $(m-M)_0 = 18.5$. This direct estimate is close to the value obtained by the use of the Tully-Fisher relationship ($D = 18.4 \pm 2.2$ Mpc), but very different from Sandage's values of $D = 27.7$ Mpc based on a calibration of the diameters of Sc I galaxies.

Cepheids in NGC 4571

The ground-based sample of Pierce *et al.*⁴ contained certain identification of two classical Cepheids and one suspected one in NGC 4571. This galaxy is located at less than $2^\circ.5$ from M 87 as projected in the sky. Further it shows evidence of significant HI stripping. Its kinematics shows that it is close to the cluster core and most likely is in orbit around the dynamical centre of it. The periods of Cepheid variables range from 50 to 90 days and again define a linear relation with their mean R magnitudes, the slope of which is the same as found in the LMC Cepheid sample. The distance $D(\text{N4571}) = 14.9 \pm 1.2$ Mpc.

Observations of classical Cepheids in two of the spirals of the Virgo cluster seem thus to support the so-called short distance scale to this cluster ($D_{\text{Virgo}} \sim 15 \pm 2$ Mpc). But before deriving H_0 based on these distances the measurement of velocities of the cluster galaxies should be critically examined to infer the true Hubble velocity of the cluster.

Cosmic velocity of the Virgo cluster

Our local region of space, cosmologically speaking, shows a complexity of motion that needs to be disentangled before the true Hubble flow of the Virgo cluster can be estimated. It has been known for several years now that our own Local Group has a Virgocentric flow of a few hundred km s^{-1} . Observations of the 3 K Cosmic Microwave Background (CMB) show a dipole anisotropy, implying a motion towards its warm pole, but the direction does not coincide with the direction of the Virgo cluster in the sky. Some years ago it was postulated that there is a Great Attractor (GA), a large hidden clump of mass towards which all galaxies in the local superbubble (defined as a cell within $V_r \leq 6000 \text{ km s}^{-1}$) are attracted. More recent and extensive data by Mathewson *et al.*⁷ have demolished the GA concept and it is now well established that the nearly bulk peculiar motion of the local superbubble gradually peters out beyond 6000 km s^{-1} , merging gradually into the unperturbed Hubble flow.

In the currently accepted picture of the dynamics of

the local region consistent with all kinematic data, there are two components to the motion of the Local Group relative to the frame of CMB: (i) the perturbation of the free expansion of it from the Virgo region due to the mass of the Virgo cluster complex and (ii) the large scale nearly bulk motion of the local bubble of size $V_r \leq 6000 \text{ km s}^{-1}$ relative to the CMB, carrying the Local Group and the Virgo complex with it. Figure 1 displays a vector diagram of the velocities in the local region, adapted from Sandage and Tammann⁸. These authors assert that the peculiar dipole motion toward the CMB within the Local Supercluster and beyond is so small that it can be neglected in the determination of H_0 .

The most direct method of determining the true Hubble velocity of the Virgo cluster is to use the Hubble diagram with redshifts of remote clusters in the kinematic frame of the CMB plotted against the relative distance of these clusters with respect to the Virgo cluster expressed as a difference in their distance moduli. This is a line with a slope of 5. An extrapolation to zero in abscissa then gives the cosmic redshift at the distance of the core of Virgo cluster, devoid of all streaming motions. In this way Sandage and Tammann⁸ (*op. cit*) estimate the true Hubble velocity of the Virgo cluster to be $1179 \pm 17 \text{ km s}^{-1}$.

Hubble parameter H_0

There are two independent ways of obtaining H_0 from the recent measurements of the distance modulus to the two spirals. It is assumed, of course, that both the spirals belong to the Virgo cluster and are not foreground or background objects.

(1) Using the result, $V_{\text{Virgo}} = 1179 \text{ km s}^{-1}$, we find $H_0 = \frac{1179}{17} = 69 \text{ km s}^{-1} \text{ Mpc}^{-1}$ based on the distance to M 100 and $H_0 = \frac{1179}{14.9} = 79 \text{ km s}^{-1} \text{ Mpc}^{-1}$ from the distance to NGC 4571.

(2) Using the argument from the section 'Distance

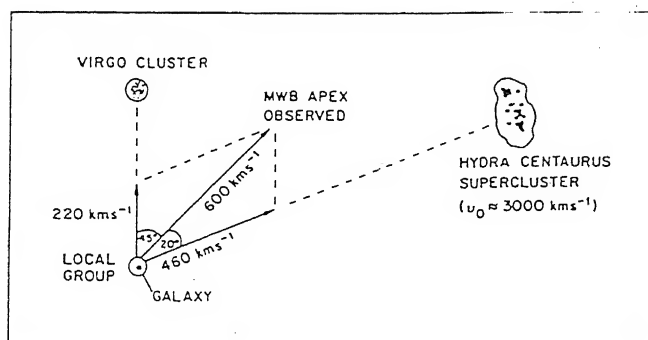


Figure 1. Schematic diagram of the combination of the 'infall' of the Local Group toward Virgo of 220 km s^{-1} and the 'infall' of the 'local cell' of size $\sim 6000 \text{ km s}^{-1}$ (including the Virgo complex and the Local Group) toward Hydra-Centaurus at 460 km s^{-1} , giving an apparent motion of the Local Group of 600 km s^{-1} toward the warm pole of the CMB.

estimates to the Virgo cluster', described above, that the relative distance $D(\text{Coma})/(\text{Virgo})$ is very secure and that its value is 5.5, we obtain $D(\text{Coma}) = 94 \text{ Mpc}$ and 82 Mpc respectively from the two distance measurements and then using $V_{\text{Coma}} = 7,100 \text{ km s}^{-1}$ to reach $H_0 = 75 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and $87 \text{ km s}^{-1} \text{ Mpc}^{-1}$.

Since there is still disagreement on the true cosmic velocity at the distance of the Virgo cluster, while as stated before the various methods of obtaining distances yield reliable results for the relative distance of the Coma cluster to the Virgo cluster, the second option of determining H_0 would seem to be more prudent. Thus direct measurement of the distance to the Virgo cluster appears to indicate that the true value of the Hubble parameter is close to $80 \text{ km s}^{-1} \text{ Mpc}^{-1}$, implying that the Universe is no more than 12.5 Gyr old if its expansion has been uniform in the past.

Disagreement with estimates based on SN Ia

Although great confidence is placed on the estimate of distances using classical Cepheids as the standard candles, secondary calibrators still occupy a very important place in setting up the extragalactic distance scale. Beyond the Virgo cluster it is the use of these secondary calibrators which lead to any distance measurement at all. Among the secondary calibrators, SN Ia occupies a unique position. These supernova are extremely luminous as point sources and they occur in all kinds of galaxies. Their observed light curves in all these galaxies appear to be remarkably uniform. The shape of the light curve is theoretically well understood. According to the currently accepted view, these supernovae are thermonuclear explosions of white dwarfs which accrete matter from a binary companion and ignite carbon in their degenerate cores which then disrupts the star completely. The characteristic light curve shape is theoretically reproduced by trapping and thermalization of the decay products of radioactive ^{56}Ni and ^{56}Co in $1.4 M_{\odot}$ of ejected matter. This model brings with it a self-calibration of the peak luminosity. An impressive amount of theoretical work has been done over the years on SN Ia explosions and these lead to a computed peak luminosity in the neighbourhood of $M_B = -19.5$. Empirical calibrations of SN Ia luminosities have depended upon observations of these supernovae in various galaxies whose distances are estimated by other methods. Those supernovae for which prepeak B and V light curves are available had their peak luminosities calibrated with reasonably good accuracies.

Since two well-known SNe Ia occurred in galaxies near enough for the HST to look at their Cepheid population, an equally important project for HST was to use the Cepheids in these galaxies to calibrate the peak luminosity of SN Ia. Saha *et al.*^{9,10} observed a number of Cepheids in NGC 5253 and IC 4182 the

host galaxies of the well observed SN 1972E and 1937C respectively. Using the Cepheid distances to these galaxies the peak luminosities of the SNe Ia were calibrated in B and V. The final values of $M_B(\text{max}) = -19.65 \pm 0.13$ and $M_V(\text{max}) = -19.60 \pm 0.10$ are in good agreement with the theoretical self-calibrated values.

To obtain the Hubble parameter from SN Ia measurements it is customary to use the SN Ia Hubble diagram which is a plot of the velocities of receding galaxies versus SN Ia V magnitudes in them. From Sandage and Tammann⁸ one obtains: $\log V_r = 0.2V + 0.658$, and hence $\log H_0 = 0.2M_V(\text{max}) + 5.658$. The value of H_0 obtained from these measurements is $52 \pm 9 \text{ km s}^{-1} \text{ Mpc}^{-1}$. This value disagrees with the high value obtained directly from the Cepheid variables. Thus there is discrepancy between the Cepheid measurements and SN Ia measurements and it is sure to focus attention on the estimates of SN Ia magnitudes used in producing the SN Ia Hubble diagram and also on the suitability of SN Ia as standard candles.

Implications of the new measurement

The HST is carrying out further measurements of classical Cepheid variables in other member spiral galaxies of the Virgo cluster and though it may take some more time to pin down the distance to the Virgo cluster with much higher accuracy, it may not be totally out of place now to discuss the implications of a high value of H_0 , should it come to stay.

The Hubble parameter in a simple sense determines how big the Universe is and how old it is. The inverse of it, which has the dimensions of time, characterizes the time scale for the expansion of the Universe at any epoch. Thus if the Universe expanded uniformly, $1/H_0$ would be the age of the Universe in Gyr (1 Gyr = 10^9 yr). However, since the Universe contains matter, it is natural to assume it has decelerated since the expansion began and in the most popular cosmological model, known as the Einstein-de Sitter model (also the Standard Model) with $\Omega_0 = 1$ and $\Lambda = 0$, the age is $2/3 H_0$. The question of the age is thus intimately related to three parameters H_0 , Ω_0 and Λ . Ω_0 is a dimensionless measure of the density of matter in the Universe and determines the deceleration of its expansion. Formally, $\Omega_0 = 8\pi G\rho/3H_0^2$, where ρ is the density of matter and G the universal gravitation constant. Λ is known as the cosmological constant, first introduced by Einstein in 1917 to obtain static solutions to the field equations of general relativity as applied to the Universe. It represents the energy density of the vacuum. So far it has not been possible to obtain either Ω_0 or Λ from observations. While all cosmological models (Friedmann Universes) set $\Lambda = 0$, a variety of arguments and deep philosophical convictions have led to the belief that we live in a Universe where $\Omega_0 = 1$ (see ref. 11 for a detailed dis-

cussion of these premises). Since visible matter perhaps makes up for only 1–5% of the total density required for Ω_0 to be unity, it has been commonly assumed that most of the matter in the Universe is dark or invisible. Investigation into the nature and distribution of this dark matter is one of the most active fields of research today. The measurement of H_0 described earlier therefore leads to $T \approx 8$ Gyr.

There have been independent estimates of the age of the Universe. These are based on the estimates of the ages of the constituents of the Universe. Amongst the oldest objects in the Galaxy are the globular clusters. Their ages have been determined by a combination of theory and observation related to the evolution of stars. The current best estimates indicate that many of these globular clusters are at least 16 ± 2 Gyr old. This result is in conflict with the age of the Universe quoted above. Probably some uncertainties lurk in the background of the otherwise highly successful theory of stellar evolution requiring these ages to be revised downward but the general consensus is that it will never be to the extent of a factor of two that is required by the new estimates of H_0 . If the assumption of $\Omega_0 = 1$ were relaxed and only the value of it consistent with early nucleosynthesis (abundances of D, ^3He , ^4He) and the virial masses of clusters of galaxies were adopted, which is a number between 0.1 and 0.2, even then the age of the Universe could hardly be increased beyond 11 Gyr for an $H_0 \sim 80 \text{ km s}^{-1} \text{ Mpc}^{-1}$. The only choice that remains, therefore, is to abandon the assumption of $\Lambda = 0$. If a positive cosmological constant were allowed, the age of the Universe can be lengthened sufficiently to accommodate the ages of globular clusters and of oldest stars.

Thus the implication of the new measurement of H_0 seems to be rather profound and would indicate that our preferred cosmological model needs fundamental revision. Further it may open up new directions of research and spur the observational quest for the value of Λ .

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Elucidation of secondary structures of peptides using high resolution NMR

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High resolution two dimensional NMR spectroscopy has become one of the important tools in the elucidation of three dimensional solution structure of fair-sized biomolecules. In this article we summarize the application of 2D NMR techniques to unravel the structure of small proteins and peptides. The basic theme is the systematic identification of the small number of secondary structural motifs such as α -helices, β -sheets and β -turns present in polypeptides using their through bond and through space fingerprints in the 2D NMR correlation maps. The method is illustrated using a 32-residue peptide which corresponds to a calcium-binding domain in the protein calmodulin.

AMONG the various spectroscopic methods that are employed in the elucidation of the three-dimensional structure of large biomolecules such as infrared¹ (IR), Raman², fluorescence³, circular dichroism (CD)⁴ and nuclear magnetic resonance spectroscopy⁵⁻⁹, only NMR has become one of the most sought after techniques because of its several unique capabilities. The NMR technique which started off as a physicists' tool for measuring magnetic moments of nuclei in the fifties, today finds immense use in the biological sciences and diagnostic medicine¹⁰⁻¹³. Because of its power to reveal information on the three-dimensional conformation of large molecules in solution, NMR has become a complementary technique to X-ray crystallography. For X-ray crystallography, however, one needs single crystals of optimal size. In contrast, NMR can give information on the macromolecules even when these *tumble freely* in solution, and this has made it an important technique for the understanding of structure activity correlation in biomolecules. This is very useful since many bioactive proteins and sugars are rather difficult to crystallize. Good structural correspondence between X-ray and NMR results has been obtained in a number of globular proteins¹⁴⁻¹⁶, although instances are known where significant differences have been noted^{17,18}. Another advantage of the NMR technique is that biomolecules can be studied at a simulated environment characterized by a specific pH, temperature, presence of ionic substances, etc. so that one is actually addressing the sample in physiological environment. Coupled with theoretical modelling¹⁹⁻²³ the NMR technique has provided us with a wealth of information on

protein folding^{24,25}, denaturation^{26,27}, intermolecular interactions^{28,29}, etc. It is now well established that the activity of a biomolecule critically depends on its three-dimensional conformation^{30,31}. It is known that the secondary and tertiary structures are dependent on the solvent medium, temperature, pH, presence of ions, etc. One of the major areas of research endeavour in molecular biology is 'protein folding', which is a fascinating and intriguing subject. Since the biological activity of proteins and polypeptides depends critically on the topography of the molecule apart from polarity and hydrogen bonding complementarity, it is important to get a first hand knowledge of the stereochemistry of the active site before one can start understanding biological (enzymatic) catalytic processes. This is because a transmembrane protein for example, may adopt different conformation as it traverses through the lipid membrane from the extracellular or intracellular region. It is also known that many of the enzyme proteins, which get activated only in the presence of certain metals, have different structures in the metal-bound and metal-free state. A myriad of one and two-dimensional (even higher dimensional) pulsed NMR techniques have been developed in the recent past³²⁻³⁴. The multi-dimensional techniques³⁵⁻³⁷ in particular, have helped in the increase in resolution needed for studying large biomolecules, by spreading the frequency in more than one dimension. In this process these methods also establish unique through-bond and through-space connectivities. With the current limit of supercon magnet reaching 750 MHz for ¹H, biomolecules up to a molecular weight of 20 kD can be studied.

Compared to X-ray in which the position of heavy atoms is determined around 2 Å resolution, in NMR distances between 2 Å and 5 Å unit can be resolved. The quantification is rather difficult due to possible motional effects leading only to an *average* distance information. In spite of this incomplete determination of internuclear distances, three-dimensional structures obtained from NMR agree well with the corresponding crystal structures, although significant differences between solution and crystal structures have been observed in many instances. The reason for the success of the NMR method is that the three-dimensional structures of proteins are governed by the general principles

of organization in which a few limited secondary structural motifs play an important role. NMR is uniquely adopted to detect the presence of these secondary structures through NOE (*vide infra*). In fact, the detection of a very long range interaction (through space connectivity between residues far apart in the sequence) reveals specific folding of the chain to organize the secondary structures. Normally the distances are estimated by comparing the NOE cross peak intensities from a 2D NOESY spectrum⁸. The $\langle r^{-6} \rangle$ dependence of the cross peak intensity will greatly reduce the relative error in the estimation of the distances which is six times lower compared to that of intensities³⁷.

In this article we describe the use of 2D NMR techniques in the elucidation of the three-dimensional structures of polypeptides. A brief description of the methods involved in the structural elucidation of peptides is illustrated by high resolution NMR studies on a synthetic 32-residue peptide corresponding to the first calcium-binding site of the protein calmodulin.

The severe overlap of resonances places an upper limit of ≈ 10 kD on the molecular weight of peptides that can be studied by 2D NMR. As the molecular weight increases, further spectral simplification would require adopting 3D techniques, which improve the resolution. Often site selective isotopic substitution can enormously simplify the interpretation of spectra^{38,39}. If the protein aggregates in solution, difficulties arise due to the broadening of the lines. A minimum of 1–2 mM of the protein itself is required to get the optimal S/N ratio in the spectrum. Most of the strategies developed for structural elucidation of proteins are also applicable to polypeptides.

2D NMR spectroscopy

In high resolution NMR, two major parameters, the chemical shifts and the scalar coupling constants, are needed to characterize a molecule. These give information as to the nature of the functional groups and the coupling patterns in the molecule. In addition, the nuclear Overhauser effect provides information regarding the spatial proximity of non-bonded nuclei. A complete information on the spin pattern and the through space connectivities of a molecule, should in principle, lead to the elucidation of the three-dimensional structure. However, as the molecular weight increases, the spectrum becomes complex due to severe overlap of spectral features. Therefore using 1D NMR spectroscopy, unequivocal evaluation of the spectral parameters is difficult. This becomes even more formidable when one addresses the problem of fair sized biomolecules. Methods have therefore been developed to increase the resolution of the spectrum, by spreading the same into a second dimension and in some cases even to a third dimension. The idea of a

2D strategy in NMR experiment was first suggested by Jeener⁴⁰. Later various groups contributed to the development of a number of 2D techniques^{32,41–49}.

The normal 1D NMR spectrum (whether CW or FT) is a 2D representation with one axis representing the chemical shift and the second axis, the intensity. A 2D spectrum consists of two axes representing frequencies and the third axis, intensity. 2D NMR spectra are generated using time domain techniques wherein the spin system is subjected to pulses and time intervals, and typically consists of four time sectors labelled *preparation* (P), *evolution* (E), *mixing* (M) and *acquisition* (A) (Figure 1). The preparation consists of applying an rf pulse to generate non-equilibrium magnetization. The system is allowed to evolve during the evolution period, t_1 , under suitably *tailored* Hamiltonians. The third period, mixing, which is not mandatory for all 2D experiments, may consist of suitable pulses for effecting magnetization transfer, cross relaxation, chemical exchange, etc. The acquisition period, t_2 , during which the signals are observed, is free of pulses except in some heteronuclear situations where it may consist of decoupling of one of the spins. The total time duration from preparation to the start of acquisition should be much less than the spin-spin relaxation time of the system so that it *remembers* the previous history and retains all the phase memories.

Generally, in 2D experiments a set of 1D data are collected by systematically varying the time interval, t_1 , during the evolution period. The data thus acquired at the detection period, $S(t_1, t_2)$, are first Fourier-transformed

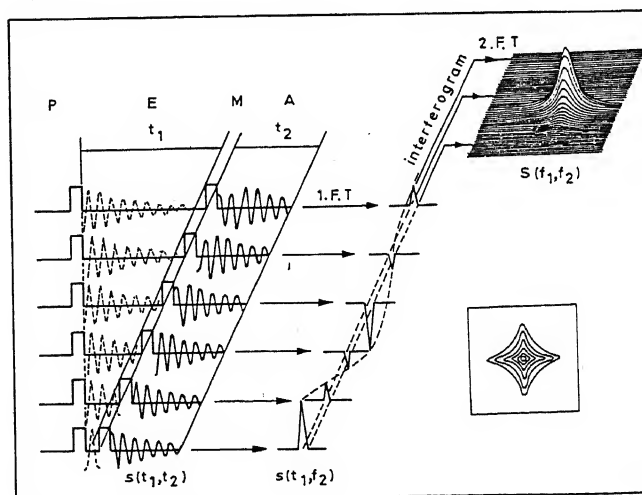


Figure 1. Schematic representation of 2D NMR. The four time periods are preparation (P), evolution (E), mixing (M) and acquisition (A). The signal $S(t_1, t_2)$ is acquired as a function of two time variables t_1 and t_2 . FT with respect to t_2 gives the interferogram $S(t_1, f_2)$ which upon FT with respect to t_1 gives the 2D spectrum $S(f_1, f_2)$. This can be represented either in a stack plot (inset on the top) or a contour plot (bottom inset).

along the t_2 axis. The intensities of signals, $S(t_1, F_2)$, vary as a function of t_1 , due to modulations of phase/amplitude depending upon the operative Hamiltonian. These data are then transposed and Fourier-transformed along the t_1 direction, to give the normal 2D spectrum, $S(F_1, F_2)$. A 2D spectrum consists of three types of peaks. *Diagonal* peaks which occur at the 45° diagonal of the 2D map, correspond to the magnetization which does not undergo any transfer and was not affected by pulses, delays, etc. between the preparation and acquisition period. (A projection of the diagonal peak on either axis represents the normal spectrum.) *Off diagonal* peaks or cross peaks occurring at coordinates (ω_i, ω_j) with the corresponding symmetry related peak occurring at (ω_j, ω_i) represent connectivities either through bond or through space. Axial peaks occur at $\omega = 0$ along the ω_1 dimension. These correspond to creation of fresh transverse magnetization signals that arise from the Zeeman magnetizations that recovered *via* spin-lattice relaxation during the t_1 period and as such do not have any useful information and can be removed by suitable phase cycling. 2D spectra are either represented in a stacked mode or in a contour form. Normally contour representation is preferred, as this is more informative, where the peak intensity is proportional to the number of contours encircling the peak position (see Figure 1).

Unlike in 1D spectra, it is not normally possible to expect pure absorption mode line shapes in either or both coordinate axes of the two frequency domains. Usually, a typical 2D NMR peak has a *phase twisted* line shape, with characteristic long tails extending well beyond the position of the lines. This is because, the 2D line shape function is often a mixture of absorption and dispersion in both the dimensions. Conventionally, the practice was to plot the absolute value mode spectrum $(u^2 + v^2)^{1/2}$ to avoid distortions in contour display. The FIDs are often weighted by suitable apodization functions⁴² to get maximum resolution. However, absolute value mode spectrum always leads to poor resolution, hence most of the 2D sequences and the mode of acquisition have been modified in recent times so as to produce pure absorption mode line shapes in both frequency domains⁵⁰.

The three most important homonuclear 2D NMR experiments are chemical shift correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY) and nuclear Overhauser effect spectroscopy (NOESY). These methods involve the use of multipulse sequences, which manipulate the spins depending upon the internal Hamiltonian as well as the dynamics related to relaxation. Since nuclear spin systems are finite dimensional, the quantum mechanics of spin dynamics can be evaluated exactly^{32,33}. In this connection it is possible to use, the Cartesian spin operators such as I_x, I_y, I_z and their products such as $I_x S_z, I_x S_x$, etc., as a representation of the density

operator⁵¹. It is possible to precisely describe the state of the spin state and the fate of the same during the pulse sequences and the nature of the acquired signal using the density operator formalism.

Correlation spectroscopy

In COSY⁴² the system is subjected to a two-pulse sequence (Figure 2). The first pulse creates transverse magnetization of all the spins. These evolve in the laboratory frame with their characteristic chemical shift (δ) and spin-spin coupling (J). Those magnetizations corresponding to J coupled spins produce signed antiphase magnetization⁷ during the evolution in t_1 . The second pulse acting on these antiphase magnetizations effects a magnetization transfer coherently among coupled partners with the transfer rate being proportional to $J/2$. A 2D Fourier transformation of the acquired signal, therefore, produces off-diagonal peaks at coordinates

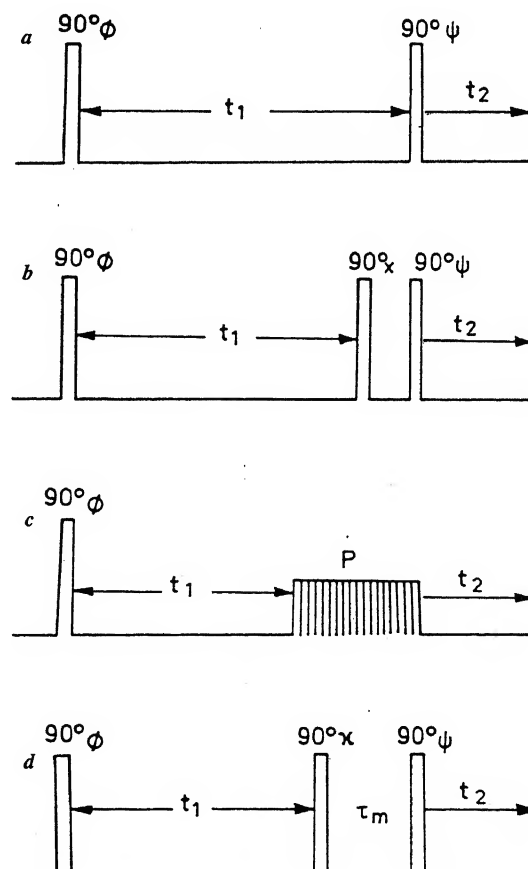


Figure 2. Pulse sequences for (a) 2D COSY, (b) 2D DQF COSY, (c) 2D TOCSY and (d) 2D NOESY. In (c) P corresponds to a composite pulse sequence for locking the magnetization in the transverse plane. In (d) τ_m is the NOE mixing time. The phases of the various planes denoted by Greek alphabets are suitably cycled to get the desired magnetization transfer pathway.

(ω_i, ω_j), indicating that the two spins with chemical shifts ω_i and ω_j are connected through bond. COSY establishes connectivity between all directly coupled spins in the system. A detailed description of the mechanism of J connectivity and the phase cycling required to produce pure phase spectra are given in ref. 32.

In the normal COSY methodology described above, the diagonal peaks are of inphase dispersion lineshapes, they have long tails, often obscuring cross peaks close to the diagonal. Isolated equivalent spins such as methyl, isopropyl and *t*-butyl also produce exclusive diagonal peaks with long tails and often interfere with the identification of other cross peaks close to them. Since 2D COSY is essentially a means for establishing J connectivity it is possible to improve the spectrum by using double quantum filtered COSY⁵², DQF COSY. In this method, the second 90° pulse of COSY is replaced by a pair of 90° pulses with a very short delay in between (Figure 2). The first of these pulses generates double and higher quantum coherences only among J coupled partners, which are converted into observable single quantum coherences by the last pulse. A suitable phase cycling is used so that the magnetizations which proceed through a double quantum coherence pathway are exclusively accounted for in the t_2 period. Since solvents (water, CDCl₃, DMSO, acetone, benzene, etc.) and isolated spins [–OCH₃, –C(CH₃)₂, etc.] do not possess J coupling, signals from these are eliminated from the 2D spectrum. Further, when the experiment is carried out in pure phase mode, the diagonal peaks (which now represent only spins with J coupled partners) become quite narrow because of pure absorption line shapes and enable identification of cross peaks close to the diagonal. It is to be pointed out that in going from COSY to DQF COSY, though the resolution is enhanced, there is a decrease in sensitivity since the conversion from single quantum to double quantum and back to single quantum leads to loss of magnetization in both conversions by the creation of zero quantum and zz magnetizations⁵³.

Total correlation spectroscopy

To establish the sequential J connectivity in a molecule, an extension of COSY is used which relies on successive relay of magnetization between spins in a network. It can be shown using density operator method, just as a single 90° pulse following a preparation pulse can transfer magnetization between directly coupled partners, further evolution followed by a third pulse will transfer magnetization between spins having a common coupling partner. Such a strategy is called relayed COSY^{54,55}, and will establish the connectivity between spins having common coupling partners. A further extension of the relayed COSY which establishes complete connectivity

among spins in a consecutive network of coupled spins is known as total correlation spectroscopy (TOCSY)⁵⁶ or Homonuclear Hartmann Hahn spectroscopy⁵⁷ (Figure 2). In this method, after the creation of the transverse magnetization by a first pulse the spin system is effectively locked in the transverse plane using suitable continuous or composite pulse sequences^{58–61}. Under the circumstances, the chemical shift becomes effectively zero in the rotating frame and the spin system evolves under a strong coupling Hamiltonian, J.I.S. It can be shown that such a strong coupling Hamiltonian, depending upon the various coupling constants in the network, transfers magnetization coherently back and forth at a number of frequencies⁶², thereby establishing complete connectivity within the network.

Nuclear Overhauser effect spectroscopy^{63–67}

Through space connectivity in NMR is achieved by nuclear Overhauser effect spectroscopy. The intensity of a resonance signal in an NMR spectrum is proportional to the population difference between two energy levels of the corresponding transition and, in thermal equilibrium, is governed by Boltzmann statistics. When the equilibrium populations are disturbed (*via* rf absorption), the return to equilibrium is determined by the transition probabilities. Among the various levels in the system, the internal interactions such as chemical shift anisotropy, homo and heteronuclear dipolar coupling, nuclear electric quadrupole coupling and spin rotation interactions, are made to fluctuate due to the random Brownian motion and these bring about the transitions among the levels via radiationless processes. Among all the mechanisms that bring about the establishment of Boltzmann equilibrium, the dipole–dipole relaxation is the most important, and is almost exclusively the main cause for relaxation for protons. Under this mechanism, the transition probability depends on the strength of the local field component fluctuating at the frequency corresponding to the transition under consideration. This local field is the field at the site of a given magnetic nucleus due to the presence of other dipoles.

The sequence for 2D NOESY is given in Figure 2. The first two 90° pulses effectively invert all the magnetization to the $-z$ -axis. The non-equilibrium Zeeman magnetization tends to reach equilibrium via spin lattice relaxation brought about by dipole–dipole interaction (NOE) as explained before. In this process magnetizations get transferred between spins at a rate proportional to r^{-6} . A third pulse applied after a delay, known as the mixing time, τ_m , generates a 2D spectrum, where the cross peaks establish dipolar connectives; suitable phase cycling is used to get pure phase spectra. Because the efficiency of 2D NOESY depends critically on $\omega\tau_c$, the viscosity of the medium and hence the temperature of

measurement are the factors to be controlled, along with the optimization of τ_m . It is the capability of the NOE effect to establish magnetization transfer among dipolar partners in solution that makes the 2D NOESY the only method that can provide distance information in solution.

3D structure of peptides⁶⁸

Amino acids are the basic building blocks of peptides. All the amino acids, with the exception of proline, have a carboxyl and an amino group on the α -C atom. The individual amino acids differ in the side chain, being either aliphatic, aromatic or heterocyclic. They are usually represented by the first three letters of their names (e.g. alanine, Ala) or in short by a one-letter code (e.g. lysine, K; phenylalanine, F; etc.). The amino acids are linked together by a peptide bond to form the polypeptide chain. The peptide bond exhibits a partial double bond character and hence the six atoms, C_α -CONH- C'_α , that characterize the peptide moiety lie in the same plane. Thus the chain can only turn at the C_α atoms around the bonds N- C_α and C_α -C' corresponding to the dihedral angles ϕ and ψ . These two dihedral angles characterize the peptide backbone structure shown in Figure 3. Pro-

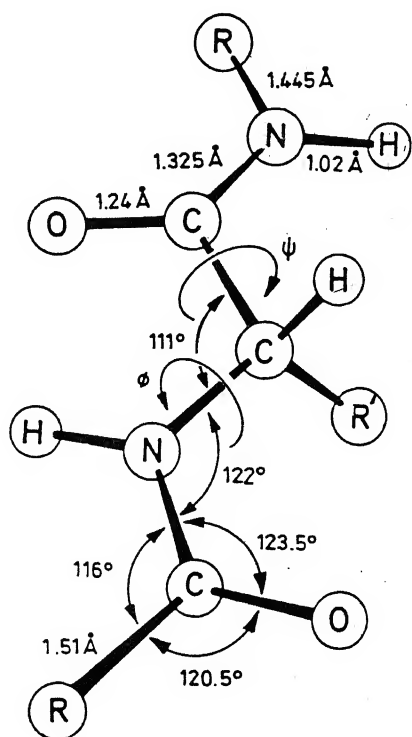


Figure 3. Dimensions of the peptide bond. The six atoms C_α -CO NH- C_α lie in a plane. The chain has the ability to turn only at the C_α atoms around the angles ϕ and ψ . Here R corresponds to the continuity of the backbone while R' represents the sidechain in any residue. (Adapted from ref. 31.)

teins are generally described by their primary, secondary and tertiary structures. The primary structure basically identifies the various amino acid residues in their exact sequence usually numbered from the N-terminal end. The secondary structure brings about additional stabilization of the molecule through intra- and inter-molecular hydrogen bonds leading to, surprisingly, a limited number of structural motifs such as α -helix, β -sheet and β -turn. Tertiary structure is brought about by the interaction among the various secondary structural elements through electrostatic, hydrophobic, van der Waals type interactions, etc. The number of protein structures solved by NMR is increasing at a tremendous pace. The advent of supercon magnets and highly sophisticated softwares to build protein models from the NMR distance data have made this one of the most important techniques^{69,70}.

Identification of secondary structures by NMR

Figure 4 shows the major secondary structural elements that are found in proteins and polypeptides. Each of these structural elements has characteristic through space connectivities, identification of which is the first step in understanding the phenomenon of protein folding^{71,72}. The characteristic folding of the polypeptide chain brings different residues in close proximity and confers the overall structure on the molecule. The quantification of internuclear distances through NOE can help in arriving at the secondary structure.

The various interproton distances that are present in a folded peptide, are generally classified as sequential, medium range and long range, depending upon the number of intervening amino acid residues. By convention, the distance between protons A and B, located in amino acid residues at position i and j is denoted by $d_{AB}(i, j)$. For example, the distance between the α proton of the i th residue and NH proton of the j th residue is denoted as $d_{\alpha N}(i, j)$. Sequential distances between the backbone protons are labelled as $d_{\alpha\alpha}(i, i+1)$ and $d_{NN}(i, i+1)$ and those between a backbone proton and β/γ protons in the side chain that are on nearest neighbour residues are labelled as $d_{\alpha/N\beta}(i, i+1)$. Medium range distances are the nonsequential inter-residue separation between backbone protons or between a backbone proton and a side chain proton, within a segment of five consecutive residues. Long range distances are those between backbone protons that are at least five residues apart⁵.

Apart from the sequential distances which characterize specific secondary and tertiary structures, the secondary structures are governed by a number of medium and long range proton-proton distances. In α -helix, the amino acid residues at position i and $i+3$ are in close proximity. The distance between NH proton of the i th residue and that of $(i+1)$ residue is short, being ≈ 2.8 Å. Also, the

distances $d_{\alpha N}(i, i+3)$, $d_{\beta N}(i, i+1)$, $d_{\alpha\beta}(i, i+3)$, are short. The presence of NOE between the above proton pairs, especially $d_{\alpha\beta}(i, i+3)$ and $d_{\alpha N}(i, i+3)$ characterizes the α -helix. β -sheets are formed when individual strands of the extended polypeptide chain come in close contact, stabilized by inter residue hydrogen bonds. These are characterized by short sequential $d_{\alpha N}$ and interstrand $d_{\alpha N}(i, j)$, $d_{NN}(i, j)$ and $d_{\alpha\alpha}(i, j)$. The presence of inter-strand $d_{\alpha\alpha}(i, j)$ (long range NOEs) is unique for antiparallel β -sheets, whereas in parallel β -sheets $d_{\alpha\alpha}(i, j)$ contacts are in the range of 4.8 Å and hence have reduced NOE. Turns are short secondary structural elements comprising four residues. These are characterized by short sequential and medium range NOEs. Table 1 summarizes the various short proton-proton distances in the secondary structural elements of polypeptides.

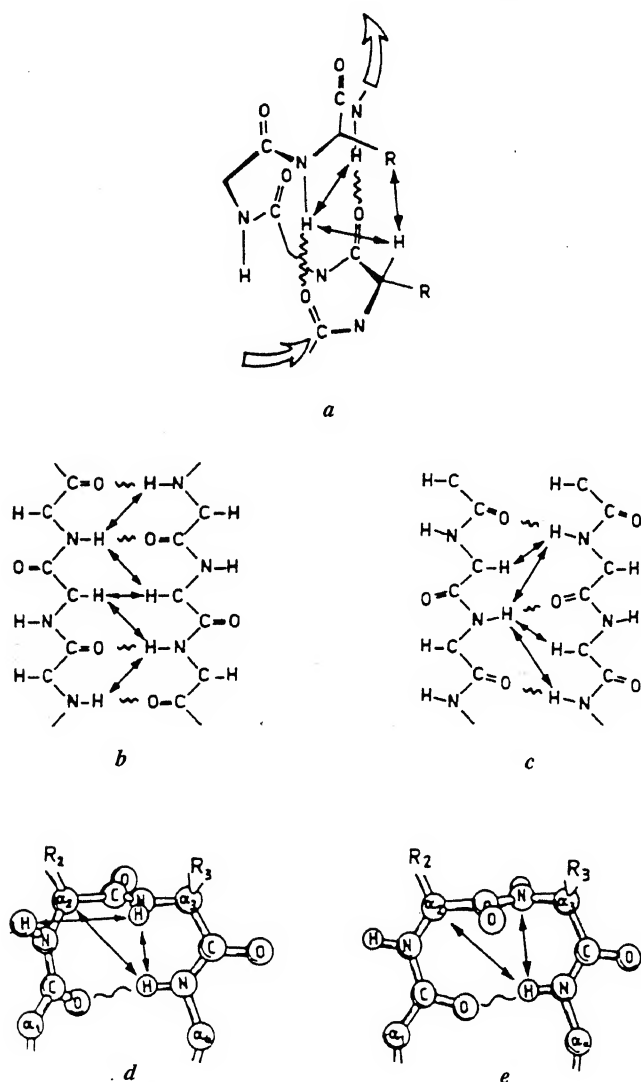


Figure 4. Schematic representation of the secondary structural elements in peptides: (a) α -helix, (b) antiparallel β -sheet, (c) parallel β -sheet, (d) β -turn type-I, (e) β -turn type-II. Short proton-proton through space connectivities are indicated by arrows and the wavy lines correspond to hydrogen bonds. (Adapted from ref. 67.)

Besides NOE, regular secondary structures are also characterized by the three bond ($\text{H}-\text{N}-\text{C}_\alpha-\text{H}$) spin-spin coupling constant ($^3J_{\text{NH}-\alpha\text{CH}}$). The torsional or rotational angle around the $\text{C}_{\alpha\text{N}}$ bond (ϕ) is related to the proton-proton coupling constant that has been extensively used to probe the polypeptide conformation in solution^{73,74}. The procedure is based on the empirical relationship between the vicinal coupling constant and the dihedral angle ϕ . This is given by a Karplus type relation⁷³

$$^3J_{\text{NH}-\alpha\text{CH}} = A \cos^2 \phi + B \cos \phi + C, \quad (1)$$

where $A = 6.4$, $B = -1.4$ and $C = 1.9$ Hz. These have been derived using BPTI as a standard and by fitting the three bond $J_{\text{CH}-\text{NH}}$ measured in solution and the corresponding dihedral angles derived from X-ray structure. The coupling constant is generally used for complementing the NOE distance data for the elucidation of backbone conformation of the polypeptide. The spin-spin coupling constant is normally derived from a high resolution COSY or DQF COSY spectra. It is difficult to derive an accurate estimate of this. The lines in both the above mentioned 2D techniques, appear as antiphase absorption peaks and when the line width becomes of the order of the coupling constant, the experimental J is an overestimate of the actual J (ref. 75).

The measured NOE as well as J cannot be interpreted uniquely unless it is known that the structure corresponds to a single conformer. When the polypeptide exists as an equilibrium of rapidly interconverting conformers, the NOE and J are the weighted average of the distances r_i^{-6} and dihedral angles ϕ_i respectively. Since the averaging processes are nonlinearly dependent on geometry, it is often difficult to correlate the NOE and J in terms of a single conformation. When more than one conformation is possible for the molecule, the NOE derived distance⁸ may lead to a structure, which may be energetically unfavourable⁹.

Assignment of the spin systems

The information about the secondary structural content can be arrived at only when the resonances have been assigned to individual amino acids. For a small peptide, spectral overlap is minimum and useful information can be obtained from 1D NMR data itself. As the number of amino acid residues increases, the complexity of the 1D spectrum increases. This is because the NH resonances occur in the region 7–10 ppm. The appearance of all the NH residues, in a fair sized peptide, in this narrow region complicates the structural information that can be obtained from various 1D techniques. This problem is circumvented by the use of 2D NMR techniques wherein the information is spread along the second

Table 1. List of short proton-proton distances (Å) found in polypeptides and proteins characteristic of the typical secondary structural elements

Distance	α -Helix	3_{10} -Helix	β	β_p	Turn I	Turn II
$d_{\alpha N}$	3.5	3.4	2.2	2.2	3.4 3.2	2.2 3.2
$d_{\alpha N(i, i+2)}$	4.4	3.8			3.6	3.3
$d_{\alpha N(i, i+3)}$	3.4	3.3			3.1-4.2	3.8-4.7
$d_{\alpha N(i, i+4)}$	4.2					
d_{NN}	2.8	2.6	4.3	4.2	2.6 2.4	4.5 2.4
$d_{NN(i, i+2)}$	4.2	4.1			3.8	4.3
$d_{\beta N}$	2.5-4.1	2.9-4.4	3.2-4.5	3.7-4.7	2.9-4.4 3.6-4.6	3.6-4.6 3.6-4.6
$d_{\alpha\beta(i, i+3)}$	2.5-4.4	3.1-5.1				
$d_{\alpha\alpha}$	2.5-4.4	3.1-5.1				

dimension. An improved spectral resolution and good signal to noise ratio is achieved by the use of high field NMR spectrometers.

Two different approaches are available for the complete assignment of the polypeptide chain. One method, sequential resonance assignment developed by Wuthrich⁵, is based on the primary structure of the polypeptide. In this method, the side chains of the various amino acid residues are assigned first and then the secondary structural elements are identified. Yet another method^{76,77}, main chain directed strategy, is based on the secondary structure of the polypeptide moiety. In this, NOE patterns characteristic of the secondary structural elements are detected without regard to the side chain type.

Through-bond correlation

To start with, the amino acid residues present in the system should be identified. All amino acid residues differ only in their side chains with their characteristic coupling network. Identification of the side chain spin system helps to know the type of amino acid residues present in the system.

Several 2D NMR experiments give through bond connectivities via J and these are COSY, DQF COSY⁷⁸, relayed COSY and TOCSY. COSY and DQF COSY give information between directly bonded spin systems with a resolved scalar coupling. Typically in a small protein or polypeptide there would be hundreds of cross peaks. A careful analysis of COSY can give information on groups of coupled protons all present in the same residue. In other words, a given amino acid will have a basic COSY pattern quite characteristic of the residue type. For the 20 common amino acids one gets ten different COSY connectivity patterns for the aliphatic region and four for the aromatic rings (Figure 5). It is

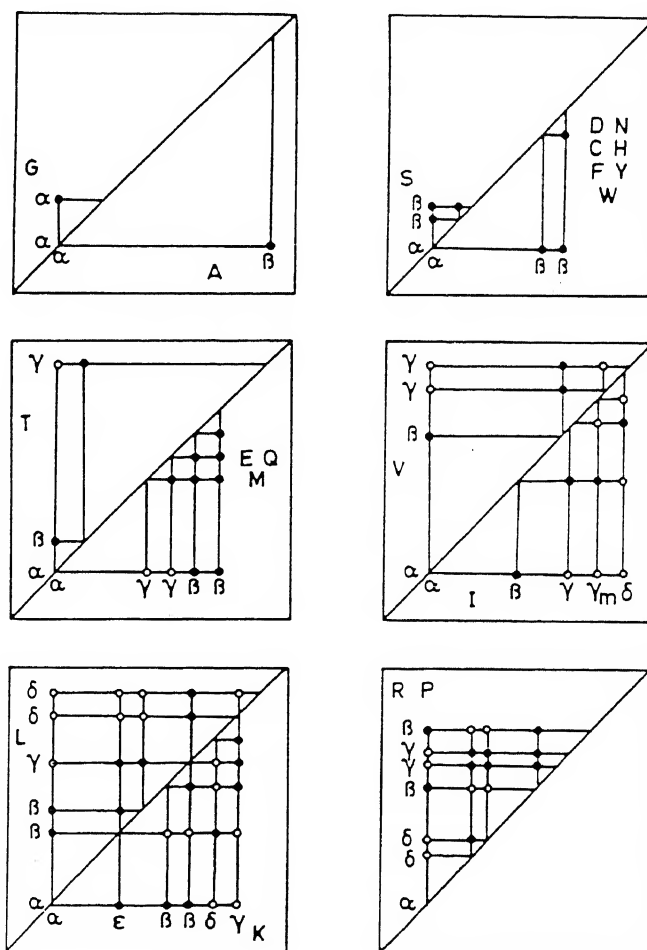


Figure 5. Patterns of connectivities through spin-spin coupling for the twenty naturally occurring amino acid residues. Filled circle cross peaks correspond to 2D COSY and additional connectivities by open circles occur in 2D TOCSY.

possible to identify the various residues by analysing the specific COSY connectivity patterns. For example, the residue Val has a COSY pattern consisting of αCH - βCH cross peaks and two βCH - γCH_3 cross peaks. An AMX spin system, on the other hand, will produce a characteristic pattern of three cross peaks. The cross peak region between the amide protons and αCH protons is the so-called finger print region, which shows a single correlation for each amino acid with the exception of glycine where the NH proton shows a correlation to two αCH protons. The only residue that can be immediately identified from this finger print region is glycine. For type-specific identification of other amino acids, connectivity information between the αCH and the side chain protons is necessary. Pro is the only residue that does not give any resonance in the finger print region. When any amino acid is repeated several times in the sequence, often the cross peak region may be fraught with severe overlap, especially when these are part of the random coil region of the peptide.

The amino acid residues like Gly, Ala, Val, Thr, Leu and Ile have unique spin systems. These residues are directly assigned through COSY spectrum. The amino acids, Asp, Asn, Cys, Ser and the aliphatic protons of the His, Tyr, Trp and Phe belong to AMX spin system. In this spin system, αCH proton is attached to two βCH protons. Although in principle COSY should help in identifying various AMX patterns, severe overlap due to the presence of large number of AMX residues, impose limitation on the identification of the particular residue type. Thus, for a residue that has NH, αCH , βCH and γCH protons, the COSY connectivities will only be manifested between NH and αCH , αCH and βCH ; and between βCH and γCH protons. Other set of residues like Glu, Gln, Met have αCH attached to two βCH protons, which in turn are attached to two γ protons. It is difficult to assign the side chain resonances of the long side chain amino residues, like Lys, due to spectral overlap. This difficulty in most instances can be resolved by supplementing COSY data with relayed COSY or TOCSY data. A typical relay pathway is from NH- αCH to β proton with larger αCH - βCH coupling and from there to γ and δ protons. In principle, therefore, TOCSY permits the correlation of all protons within a given coupling network. TOCSY with optimized mixing time, enables the complete identification of all the different amino acids, by establishing connectivity from the NH proton down to the last side chain protons. The exception is for ring protons of Phe and His residues where there is no J coupling between β protons and ring protons. TOCSY experiments can resolve ambiguities arising from coincident chemical shifts of the side chain protons. TOCSY is also known to be more sensitive than COSY and can offer better resolution. TOCSY patterns are also included in Figure 5.

In situations where degeneracy or near degeneracy of two resonances occur, multiple quantum spectroscopy will be useful. For example, a stringent test for distinguishing AMX spin systems from those of long side chains is that the βCH - βCH cross peak of an AMX systems will be completely suppressed in four quantum filtered COSY³⁴. Similar multiple quantum strategies can be used to resolve ambiguities in addition to simplifying the complexity of the finger print region.

Through space correlation

After the identification of the resonances corresponding to the individual residues, it is necessary to identify every amino acid not only by its type but also by its position in the polypeptide sequence. NOESY gives information about distances between intra- and inter-residue protons within ~ 5 Å units. Depending upon the intensity of NOE peaks, classifying them as strong, medium and weak, one can roughly deal with three ranges of distances, namely short (< 2.5 Å), medium (2.5 – 3.5 Å) and long (3.5 – 5.0 Å). To distinguish the different ranges of through space connectivities the NOESY experiment is often carried out at a number of mixing times. Often NOE cross peaks corresponding to larger internuclear distances do not show up at short mixing times.

With the unique spin topology, obtained from a combination of COSY and TOCSY, sequential connectivities between two residues of known type may provide a starting point in the sequence. This point usually corresponds to a dipeptide unit unique in the sequence. To avoid branching in the wrong direction it would be helpful to locate several di or tri peptide connectivities located along the backbone of the protein. Careful analysis of the NOESY peaks in the finger print region, when looking for specific patterns of short distances corresponding to the various motifs (α -helix, β -sheet) can, in principle, help in the assignment.

At first sight the above procedure seems quite straightforward. However, the NH- αCH protons of α -helices show little chemical shift dispersion^{79,80}, compared to β -sheet structure and often only about 50% of the residues can be identified easily. For example, two overlapping NH resonances may show intra residue and sequential NH- αCH connectivity and it becomes difficult to distinguish which of the four cross peaks correspond to which of the amide protons.

Often changes in temperature, pH and amide proton exchange when dissolved in D_2O , can simplify some of the complications. In larger peptides and proteins, several NOE experiments have to be performed by varying the experimental conditions. As was mentioned previously the secondary structures do produce recognizable finger prints in the NOESY spectrum. α -helices for example,

are characterized by a sequence of short NH–NH connectivities [$d_{\text{NN}}(i, i+1)$, $d_{\text{NN}}(i+1, i+2)$, ...] corresponding to a repetitive distance of 2.8 Å. The NH– α CH distances in α -helix give strong NOE cross peaks. In addition, weak inter-residue correlation between NH– α CH of the preceding residue $d_{\alpha\text{N}}(i, i+1)$ and to the α CH of the residue three positions earlier $d_{\alpha\text{N}}(i, i+3)$ characterize the α -helix. Other connectivities in the α -helix are $d_{\alpha\beta}(i, i+3)$, $d_{\beta\text{N}}(i, i+1)$ and relatively weaker $d_{\alpha\text{N}}(i, i+2)$, $d_{\alpha\text{N}}(i, i+4)$.

Antiparallel β -sheets will have, in addition to their interstrand α CH– α CH cross peaks, intense $d_{\alpha\text{N}}$ connectivities. Parallel β -sheets on the other hand, show sequential $d_{\alpha\text{N}}$ connectivity but rather weak interstrand α CH cross peaks. Figure 6 summarizes the various through space, NOE connectivities expected for typical secondary structural elements found in peptides and proteins.

A complementary information regarding secondary structure is obtained by quantitatively analysing the NH– α CH spin–spin coupling. Often this is done from DQF COSY spectrum with a high digital resolution in the finger print region. The Karplus relation and experimental data show that in general, this $^3J_{\text{NH}-\alpha\text{CH}}$ is in

the range of 4–5 Hz for α -helix whereas it is nearly 9 Hz for β -sheets. The various turns (type I, II, etc.) cannot be distinguished from α -helix using this information alone.

When ambiguity arises in the total assignment of the spectrum using the 2D methods mentioned above, one can resort to additional techniques such as double labelling with ^{13}C and ^{15}N of specific residues in the sequence which will aid in the assignment. This is because ^{13}C exhibits long range $^2J_{\text{C-H}}$ with NH of the next residue and its own α CH. Each amide ^{15}N has a resolved coupling with its own hydrogen ($^1J_{\text{N-H}}$) and with the α CH of the preceding residue. In peptides which lie in the intermediate tumbling region ($\omega\tau_c = 1.12$), NOESY fails and one has to resort to ROESY^{81,82} as well as the above mentioned isotopic labelling methods to arrive at the 3D structure. Another useful method is to label the side chain randomly to about 75% with ^2H . This leads to a substantial gain in the resolution because of the lengthening of the T_2 (refs 83, 84). In addition, the improved resolution is also due to the removal of spin–spin coupling between α CH and aliphatic protons. Random deuteration of the side chain leads to a gain in sensitivity in the NH–NH region of the NOESY spectrum as well, because during the mixing period of the experiment, there is a substantial reduction in the loss of magnetization due to the deuterated aliphatic chains⁸⁵.

Once the assignments of coupling network and unambiguous interpretations of NOESY spectra have been accomplished, one can resort to using this information in a distance-constrained molecular modelling study⁸⁶. However, a detailed description of the procedures will not be dealt with in this article. A schematic diagram for arriving at the most probable structures using the NMR-derived parameters as constraints is given in Figure 7.

In Figure 8 the overall summary of the structure determination of the biomolecules using NMR and NMR-assisted modelling studies is provided as a flow chart.

3D structure of a 32-residue peptide

In this last section we have taken an illustrative example from our attempts to study the 3D structure of some synthetic CaM fragments⁸⁷ corresponding to the various calcium-binding sites in isolation to see whether these have reasonable extent of secondary structures. The peptide chosen is labelled 1–1–1 and corresponds to the first calcium-binding site characterized by helix 1–loop 1–helix 1 corresponding to the residues 10–41 in the native CaM molecule, namely, AEFKEAFSLFDK DGDGTITTKELGTVMRSLGQ.

Experimental

The CaM fragments were synthesized using a modified

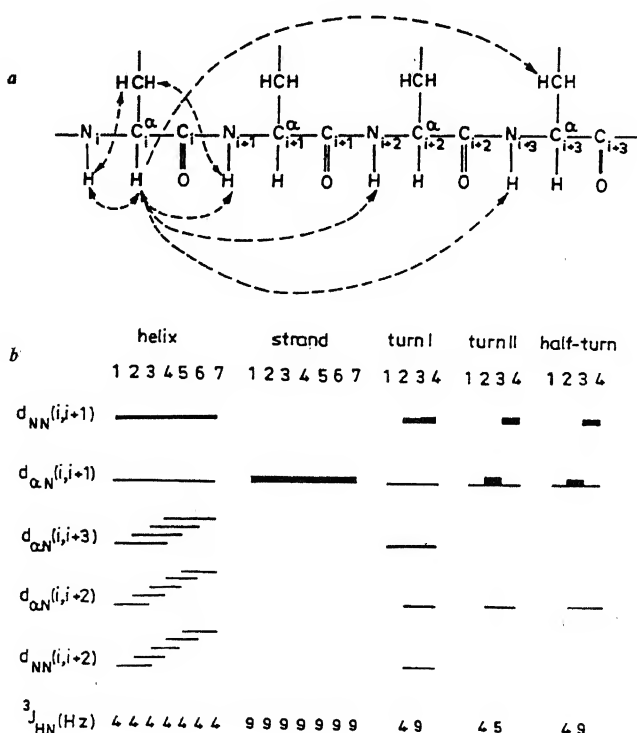


Figure 6 a, b. a, Schematic representation showing through space connectivities in peptides between protons which give NOE cross peaks. b, The various through bond connectivities in typical secondary structural elements in peptides that give NOE cross peaks. The thickness of the lines corresponds to the relative intensities of the cross peaks. The bottom row contains $^3J_{\text{NH}-\alpha\text{CH}}$ coupling constants in Hz. (Adapted from ref. 5.)

Merrifield solid phase method⁸⁸. The crude peptide was purified by reverse-phase HPLC and the purity and identity was confirmed by analytical HPLC and by amino acid sequencing. NMR experiments were carried out using a JEOL GSX 400 spectrometer and a BRUKER AMX 600 spectrometer. Measurements were made at 40°C with a peptide concentration of 4–5 mM in DMSO- d_6 . A spectral width of ~7500 Hz was used. The residual water peak in DMSO was suppressed using a presaturation pulse of 2 s duration. 2D NMR spectra, viz. DQF COSY, TOCSY and NOESY were obtained generally with 1 K complex data points, 32 transients, 256 t_1 increments and were zero filled to 2 K \times 2 K points. In some experiments the parameters were changed depending upon the requirements. Before Fourier transformation, the time domain signals were weighted by sine square or sine bell window functions in both dimensions. NOESY mixing times were optimized after doing several 2D experiments and a mixing time of 400 ms was found to be adequate. Similarly for the 2D TOCSY experiment a mixing time of 60 ms was used. All 2D NMR experiments were carried out in the phase sensitive mode and processed according to the procedure of States *et al.*

Results and discussion

The analysis of NMR spectra is based on the sequential

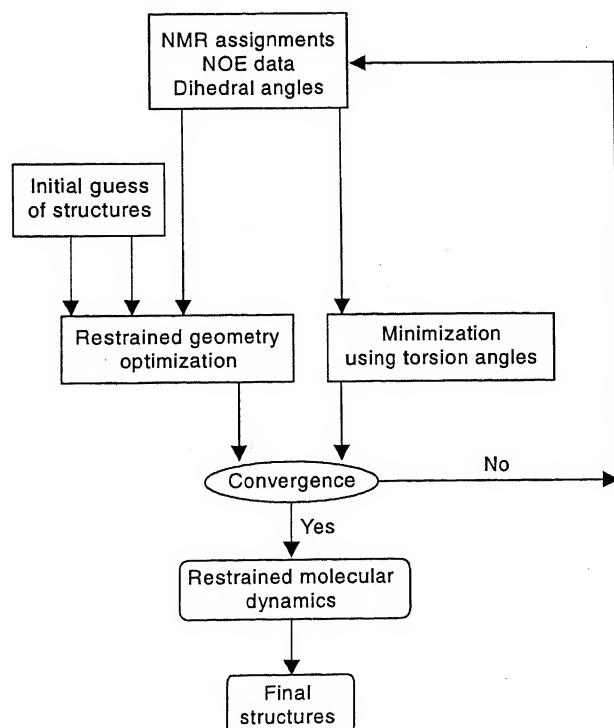


Figure 7. Flow chart for the determination of structures of peptides on the basis of NMR data.

resonance assignment procedure developed by Wüthrich *et al.*⁷⁴. This is a two-stage process. The first step involves the assignment of each cross peak in the *finger print* region to a specific amino acid type. The experiments, COSY, DQF COSY and TOCSY give information about the through bond scalar coupled spin systems. Amino acid residues with unique coupling patterns could be identified clearly whereas the other cross peaks can only be assigned to more general classes of amino acid types. Complete spin system assignment depends on the resolved coupling patterns and on the topology of the spin system under consideration. In the second stage, the cross peaks are assigned to the specific amino acid residues in the peptide sequence using NOE data⁷⁹.

Analysis of the spin systems. The peptide 1–1–1 consists of 32 amino acid residues. The DQF COSY spectrum for this peptide is shown in Figure 9a. Of the 32 residues, the finger print region clearly shows at least 22 of the NH- α CH connectivities. The remaining residues could not be located either due to severe overlap or

Steps in the structure determination of peptides by NMR

1. Sequential resonance assignment:
 - (a) Use COSY, DQF COSY, TOCSY and in some cases MQ spectroscopy to assign spin systems using through bond connectivities.
 - (b) Identify the flanking residues to a given amino acid by means of NOESY utilizing short-range connectivities.
2. Explore tertiary connectivities between non-neighbour residues through long range NOEs.
3. Estimate through space distances by quantifying NOEs into categories such as weak, medium and strong so that some distance constraints can be derived that will be useful in modelling.
4. Use the DQF COSY and other versions of COSY that allow quantitative estimates of three-bond coupling constants that will be useful to arrive at backbone torsion (dihedral) angles.
5. Look for the presence of segments of sequential connectivities that would indicate the presence of helices and probably β -sheets.
6. Finally try to arrive at the three-dimensional structure using a combination of NOE data and torsion angles in conjunction with one or more of the following approaches.
 - (a) Molecular model building packages with NOE derived distance constraints.
 - (b) Comparison with X-ray derived structures of the same peptides or homologues.
 - (c) Restrained least squares minimization in torsion angle space.
 - (d) Restrained molecular dynamics.

Figure 8.

due to the corresponding peaks being absent in the finger print region (Figure 9b). This might be due to the very low intensity arising out of a small NH- α CH coupling constant or due to the large line width. Also the overlap of antiphase components in DQF COSY spectrum leads to cancellation of most of the cross peak intensity.

The peptide 1-1-1 has 12 amino acid residues with unique spin systems, 8 of AMX type and 12 long side chain residues. There are four glycines at sequence positions 14, 16, 24 and 31. Two of the glycines are identified based on their characteristic cross peaks in the COSY spectrum. One more glycine residue, with degenerate α CH protons, is identified from the sequential NOE data. Two alanines at sequence positions 1 and 6 are identified from the COSY and TOCSY spectra. The assignment of the α CH proton is difficult due to severe overlap. One valine and all the four threonines are assigned unambiguously from the COSY and TOCSY spectra (Figure 10a, b). The identification of Ile and Leu residues is based on the appearance of the NH- δ CH₃ cross peaks. Unambiguous assignment is achieved only from the analysis of NOESY spectrum. Thus out of 15 residues of this type, 11 residues are assigned unambiguously.

The only aromatic residue present in this system is phenylalanine. Two of the three Phe are identified from the possible NOE cross peaks between the aromatic ring protons and the aliphatic protons of the same residue. NH resonance of one of the Phe is missing, but its

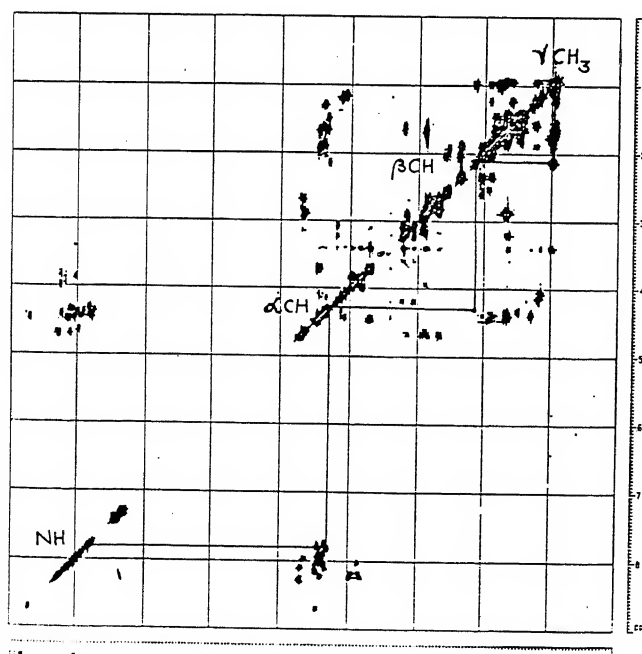


Figure 9a. 600 MHz phase sensitive DQF COSY spectrum of the peptide 1-1-1 in DMSO at 40°C. The COSY connectivities of valine are shown.

position is identified from the NOE data. Two serines are distinguished from the aspartate residues due to the low field resonances of β protons. Seven out of eight residues of this type are thus identified.

The long side chain spin systems were grouped into one class partly on the basis of chemical shift values of the β CH protons and partly on the patterns of the α CH- β CH cross peaks which are distinct from those of the AMX spin systems. The identification of the additional resonances belonging to the spin system helps in distinguishing the type of amino acid, i.e. lysines from glutamates, methionines, etc.

Sequential assignment. The sequential assignment of the peptide 1-1-1 dissolved in DMSO solution is based on the analysis of NOESY spectra with a mixing time of 400 ms (Figure 11a). The unambiguous assignment of the residues valine, glycine and threonine helps in the identification of the sequence position of the other residues. Analysis of the finger print region of the NOESY spectrum helps in the assignment of the peptide segments, Phe-3-Lys-4; Asp-11-Lys-12-Asp-13-Gly-14-Asp-15; Gly-16-Thr17; Gly-24-Thr25; Val-26-Met-27. The peak position of the Phe-3 is located on the basis

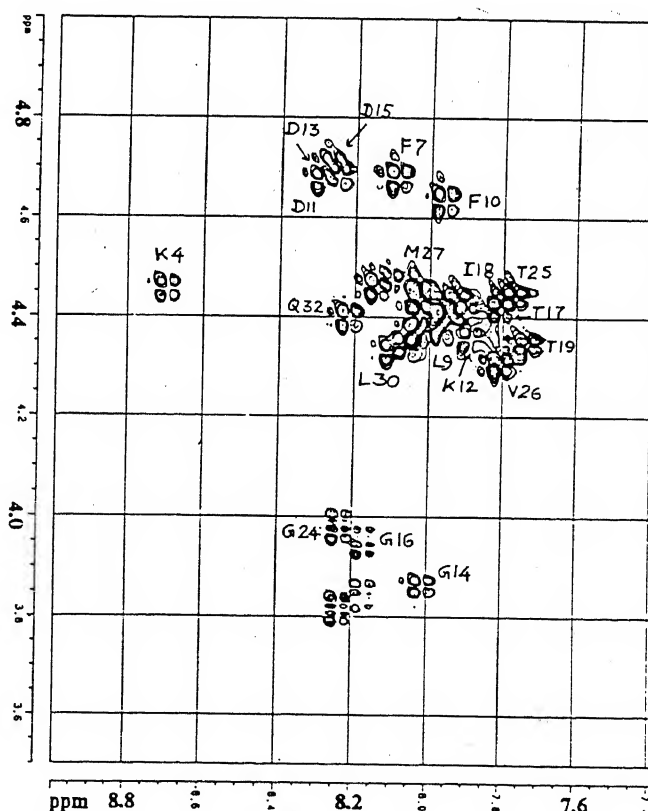


Figure 9b. 600 MHz phase sensitive DQF COSY spectrum of the peptide 1-1-1 in DMSO at 40°C, showing the expanded finger print region. The NH- α CH cross peaks have been labelled with the one-letter amino acid codes.

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of its NOE with Lys-4. The other two phenylalanines are at positions 7 and 10. The residue at position 10 is identified on the basis of the NOE cross peak between the ring proton and the γ CH proton of Leu-9. Thus all

the three phenylalanines are located clearly. Analysis of the NH-NH region of the NOESY spectrum (Figure 11 b) confirms the sequential assignment. The residue Leu-23 is assigned based on the NH-NH cross peak between

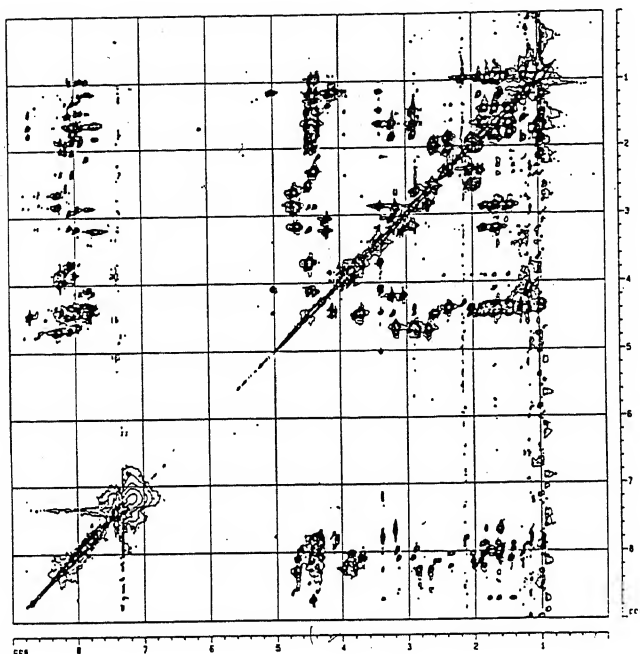


Figure 10 a. 600 MHz phase sensitive TOCSY spectrum of the peptide 1-1-1 in DMSO at 40°C with spin lock duration of 60 ms.

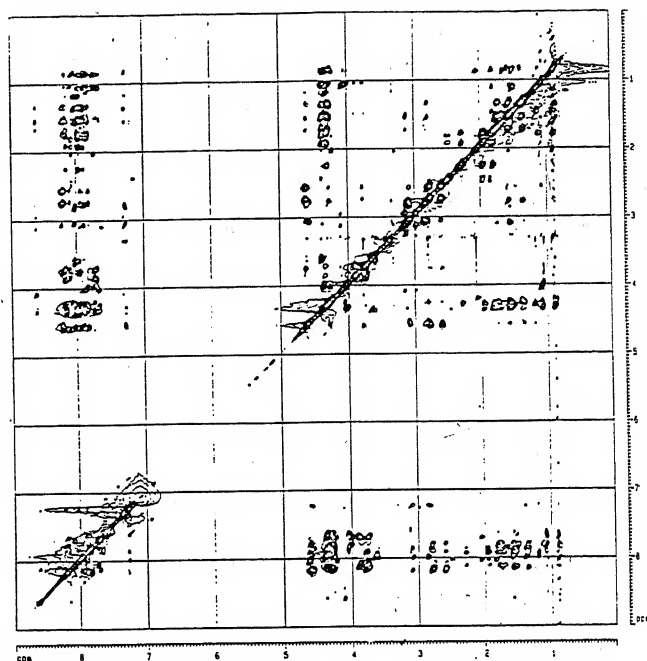


Figure 11 a. 600 MHz phase sensitive NOESY spectrum of the peptide 1-1-1 in DMSO at 40°C with a mixing time of 400 ms.

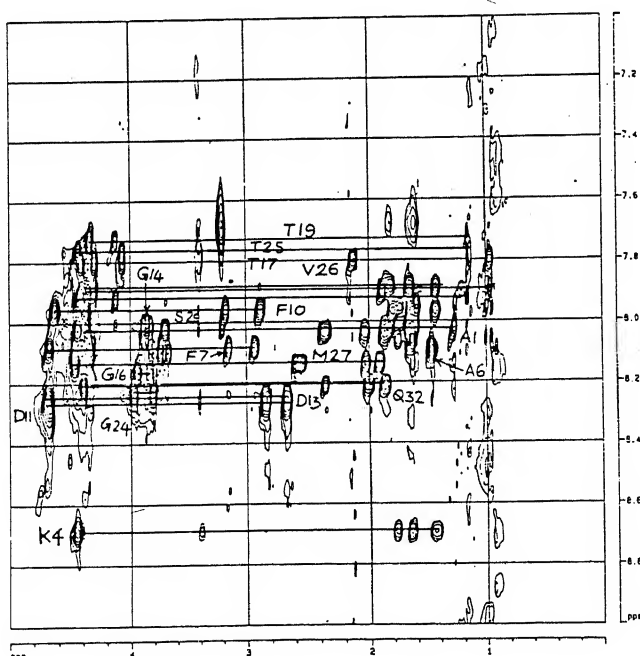


Figure 10 b. 600 MHz phase sensitive TOCSY spectrum of the peptide 1-1-1 in DMSO at 40°C with spin lock duration of 60 ms, showing a part of the TOCSY spectrum. Some of the coupling networks have been labelled.

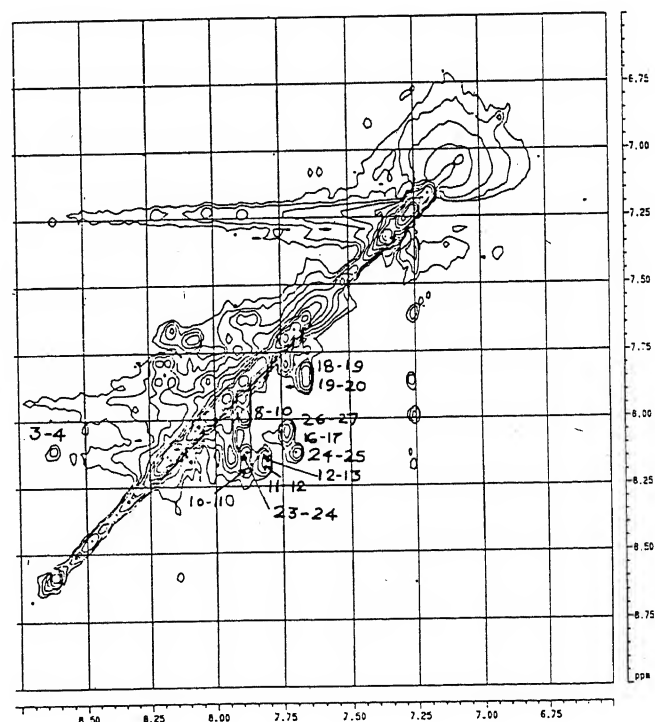


Figure 11 b. The amide region of the NOESY spectrum of the peptide 1-1-1.

Leu-23 and Gly-24. The unambiguous assignment of the two lysines, at sequence positions 4 and 12 enables the assignment of the remaining one to Lys-21. The presence of weak NH-NH cross peak between Lys-21 and another long side chain containing residue, helps in the assignment of Glu-22. Identification of the Leu-9 and Leu-23, made the assignment of the remaining leucine residue to Leu-30. A summary of through space connectivities inferred from 2D NOESY for the peptide 1-1-1 is given in Figure 12.

Secondary structural elements. The presence of strong NH-NH connectivities from the Phe-10 to Asp-15 and Gly-16 to Thr-20 indicates that the peptide exhibits helical structure in these regions. The NH resonance of all the three aspartates appears almost close to each other, making the assignment of the $\alpha\text{CH}_i\text{-NH}_{i+3}$ cross peaks between the residues, Asp-11-Gly-14; Lys-12-Asp-15; and Asp-13-Gly-16, difficult. The absence of $\alpha\text{CH}_i\text{-NH}_{i+3}$ for the peptide segment Gly-16 to Thr-20, indicates that this part is characterized by a loose helical segment. The strong $\alpha\text{CH}_i\text{-NH}_{i+1}$ and a weak $\alpha\text{CH}_i\text{-}\alpha\text{CH}_{i+1}$ between Asp-11 and Asp-15 indicate the possibility of an extended structure. Careful analysis of the $\alpha\text{-}\alpha$ region shows only three distinct binary connectivities among residues 14-15, 24-25 and 8-9. In view of this lack of contiguous connectivity it is clear that the system, although may exist transiently as an anti-parallel β -sheet, the average conformation is more like an extended structure. All these point to a possible conformational equilibrium between an extended and

helical structure. This fact is again supported by the presence of weak sequential $\alpha\text{CH}_i\text{-}\alpha\text{CH}_{i+1}$ connectivities for the residues Lys-12 to Asp-15. The presence of strong $\alpha\text{CH}_i\text{-NH}_{i+1}$ and $\text{NH}_i\text{-NH}_{i+1}$ cross peaks between the residues Phe-3-Lys-4; Gly-24-Thr-25; and Val-26-Met-27 indicates the possibility of a turn structure. The presence of $\text{NH}_i\text{-NH}_{i+2}$ and $\beta\text{CH}_i\text{-NH}_{i+2}$ connectivities between the residues Phe-7 and Phe-10 indicates the existence of a β -turn structure in this region. The end residues are not well characterized due to lack of NOE information.

Summary and conclusions

A brief summary of the approach to the elucidation of 3D structure of fair-sized peptides using high resolution 2D NMR techniques has been presented and the procedure is illustrated with a small 32-residue peptide corresponding to the first calcium-binding domain of the protein calmodulin. Whereas this part in the crystal of the native molecule has a clear cut helix-loop-helix (EF motif) configuration, fragmentation has led to a large reduction in the secondary structure. It is unfortunate that the solubility of the fragment has been so low that the experiments had to be carried out in DMSO solution. Often NMR methods are supplemented with other experimental techniques such as circular dichroism, Fourier far IR and Raman spectroscopy. The results obtained are used as input into molecular modelling and molecular dynamics calculations¹⁹⁻²³ where the NMR NOEs can be used to provide certain constraints in

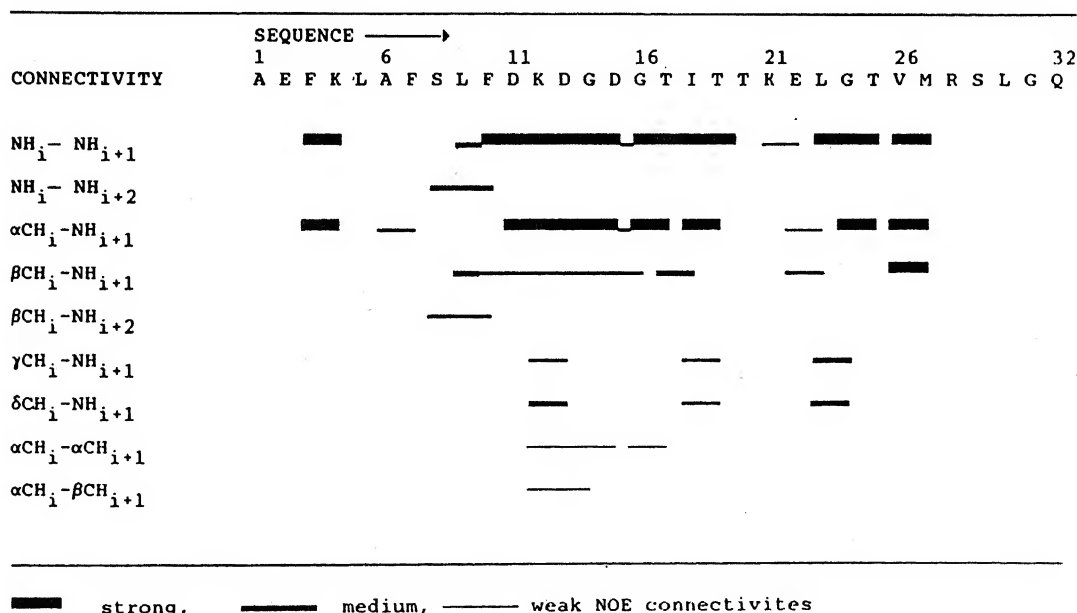


Figure 12. Schematic diagram showing the various through space connectivities derived from NOE data for the peptide 1-1-1.

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interatomic distances so as to arrive at plausible structure in an expeditious way. Caution has to be exercised in using the NOE data when one suspects rapid interconversion between conformers.

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Selected candidates will be informed latest by 11 November 1996, when the Course fee should be remitted. Details will be provided in the Selection Letter.

Patterns of distribution of macrolichens in western parts of Nanda Devi Biosphere Reserve

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A total of 76 species of macrolichens were recorded from 16 transects of 50 m × 10 m between altitudes of 2100 m and 4500 m in western parts of Nanda Devi Biosphere Reserve of Garhwal Himalayas. Forty-one of these are lignicolous species occurring on woody, 14 are terricolous growing on soil and 10 are saxicolous inhabiting rocks only. The other 11 species occur on more than one major types of substrate. Lichen species diversity is at its highest in middle altitudes between 2700 m and 3700 m where all three major substrates are simultaneously available. Lichen species diversity of Nanda Devi Biosphere Reserve appears to be under threat from deforestation and fires, as well as from loss of soil microhabitats due to overgrowth of weeds seemingly caused by cessation of summer grazing in alpine pastures.

LICHENS, the most successful of symbiotic organisms on earth, constitute the dominant life form over as much as 8% of earth's surface¹. They are amongst the most significant bioindicators²⁻⁶ besides having many economic applications^{7,8}. While there have been some systematic studies of the 697 species rich macrolichen flora of India⁹, there have been no investigations of their community ecology. We report here the first such community level study from the western parts of Nanda Devi Biosphere Reserve (NDBR) (30°30' to 30°38' N latitudes and 79°44' to 79°46' E longitudes). The entire biosphere reserve extends over an area of 2236.74 sq km in the upper reaches of Garhwal and Kumaon Himalayas spanning an altitudinal range of 1000 m to 7817 m. Our study area falls within the range of 2100 m to 4500 m above mean sea level in the landscapes of the Garhwal Himalayas with an estimated area of 500 sq km (ref. 10) (Figure 1). This rugged mountainous landscape includes crystalline rocks belonging to Vaikrita Group and lower parts of the Tethys sediments along the river Rishi Ganga¹¹. Although there are no records, precipitation is estimated to range over 1000 mm–1500 mm per year including heavy snow fall during December to March. Except alpine meadows, all other study sites are exposed to various levels of human interferences including grazing and fuel wood collection.

Methodology

Lichen communities were investigated in the western parts of NDBR over an altitudinal range of 2100 m to 4500 m during September–October 1993. Since a large part of study area falls within core zone of the reserve, administrative and logistic constraints did not allow us to sample areas above 4500 m. The sampling method involved laying down 16 transects of 50 m × 10 m in different localities covering temperate pine forest, sub-alpine birch-rhododendron forest and alpine meadows along with road-side grass and scrub at the lower altitude. A thorough search of 500 sq m transect consumed between 1 and 4 hours. Records were maintained of macrohabitat types, mesohabitat conditions with respect to exposure to sun, exposure to wind, habitat slope and humidity as well as of microhabitats of all the lichen colonies encountered (Table 1). These include three major substrates, viz. rocks, soil and wood; with further discrimination of 9 soil microhabitat types and 31 types of microhabitats in relation to species-specific wood, position on a tree and whether the wood is live or dead (Table 2). Mesohabitat levels were assigned on the basis of ordinal scaling whereas the macrohabitat types were nominally categorized¹² based on the ground vegetation. While lichens could not be sampled on trees above a height of 2.5 m, many canopy species were encountered through collection of fallen branches and twigs on the ground.

Results and discussion

Lichen flora

A total of 76 species belonging to 24 genera and 18 families constituted the macrolichen community of the western part of NDBR occurring in 204 colonies over the 8000 sq m sampled¹³ (Table 3). Figure 2 shows levels of species richness in our study area along with 8 other investigations in different parts of the country¹⁴⁻¹⁷. Evidently our sampling has yielded a higher level of species diversity in relation to the area sampled compared

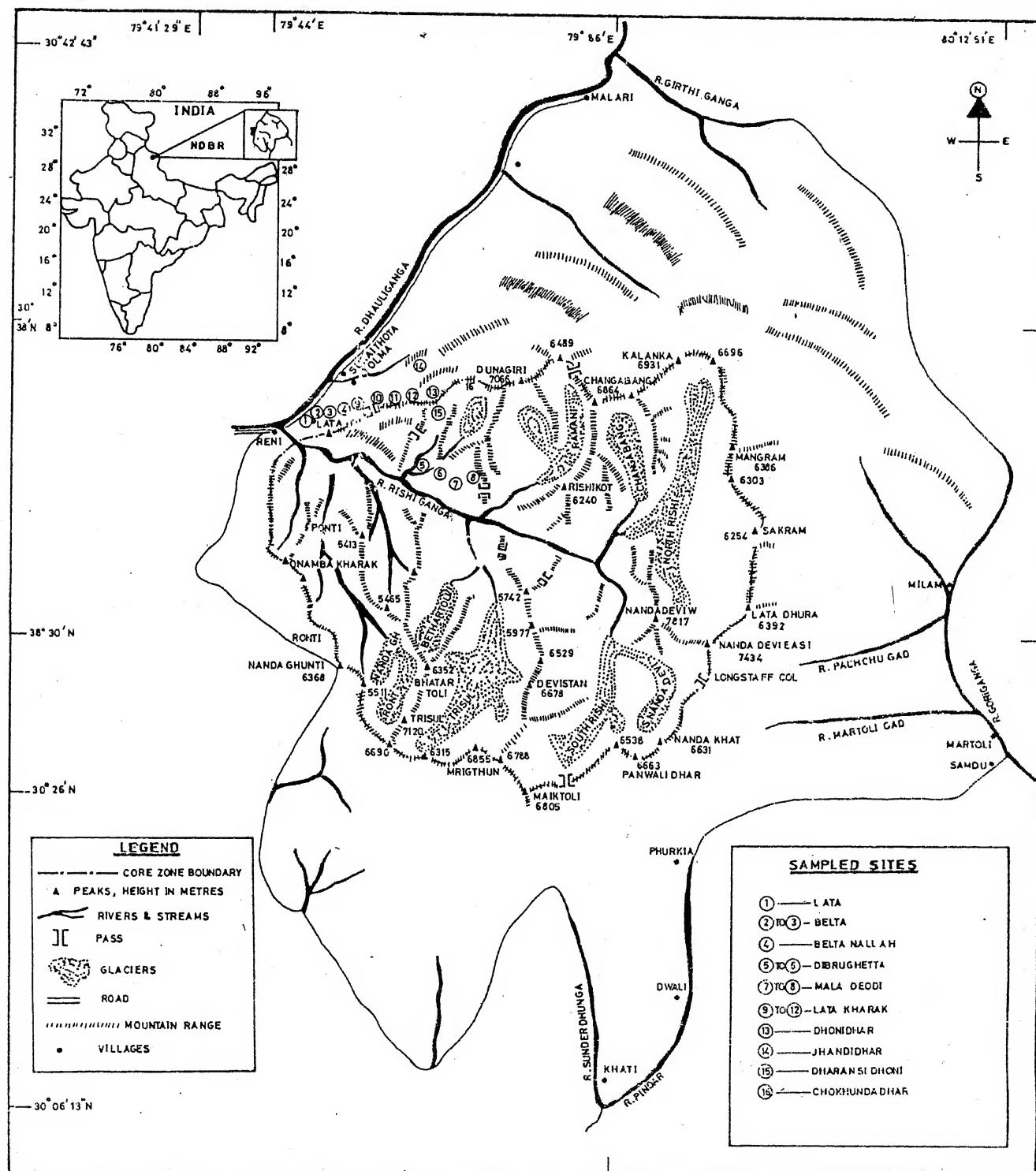
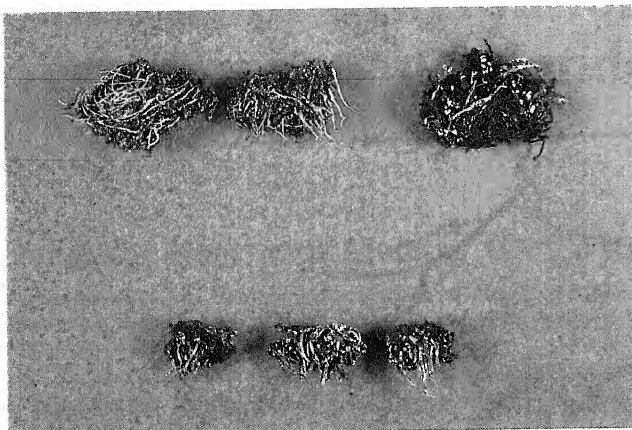
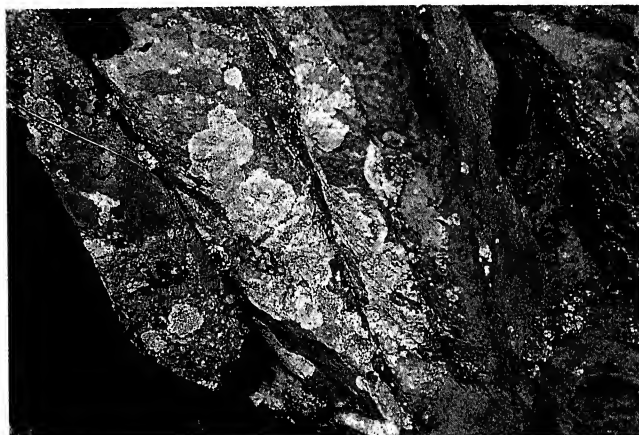


Figure 1. Schematic map of Nanda Devi Biosphere Reserve (scaled map is not available).

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Cladonia coniocraea, abundantly encountered macrolichen occurring on soil as well as wood substrates.



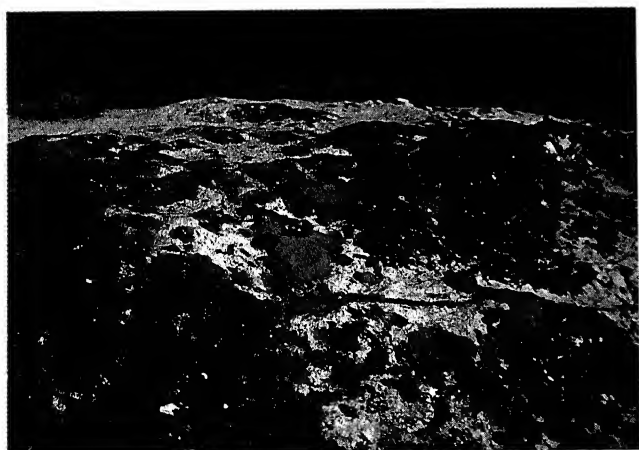
Rhizocarpon geographicum, abundantly found microlichen species only grow on rocks.



Lichens of genus *Parmelia* growing on tree trunk bark at 2500 m.



Lichens are the dominant forms of life on the exposed rocks in the higher reaches of Nanda Devi Biosphere Reserve.



Xanthoria elagans, a colourful lichen growing on rocks at 4000 m.



Birch tree supporting a luxuriant growth of longest of lichen thalli of *Usnea longissima*.

to other studies reported from India. Figure 3 shows the patterns of relative abundance of the species showing that most species were encountered in 1 to 4 colonies only. *Cladonia coniocaea* was the most abundant of all the species.

Niche-width of species computed as Shannon-Wiener index¹⁸ with respect to fine divisions of the substrates (microhabitats) is, as expected, positively correlated with

the number of colonies encountered. After correcting for this factor, *Parmelia cirrhata* emerges as a significantly broad niched species while *Dermatocarpon velterium* and *Umbilicaria indica* as two significantly narrow niched species with respect to their fine microhabitat preference (Figure 4). Nine of the 20 more abundant species occurring in four or more colonies occupy two out of three major substrates, and may therefore be

Table 1. Details of locality, macrohabitat, mesohabitat, major substrates, macrolichen abundance and species richness for the sixteen 50 m x 10 m transects

Locality	Altitude (m)	Macrohabitat	Mesohabitats				Major substrates	No. of colonies	No. of species	Significance
			Sn	Wn	Slp	Hm				
Lata	2100	Road side scrub and grass	3	3	1	2	Soil, rocks	3	1	
Belta	2500	Kail*-dominated habitat	1	1	2	2	Soil, rocks, wood	20	12	
Belta	2700	Raga*-dominated habitat	1	1	3	2	Soil, rocks, wood	27	21	+
Belta Nallah	2800	Raga*-Bhojpatra*-Thuner* dominant habitat	1	1	3	2	Soil, rocks, wood	12	9	
Dibrugetta	3300	Rocky rivulet bank	2	2	2	3	Soil, rocks	11	6	
Dibrugetta	3400	Raga*-Bhojpatra* dominant habitat	1	1	3	2	Soil, rocks, wood	20	16	+
Mala Deodi	3500	Scrubby patch with a Bhojpatra*	1	1	1	2	Soil, rocks, wood	8	5	
Mala Deodi	3500	Grassy patch dotted with Bhojpatra* and shrub	2	2	2	2	Soil, rocks, wood	8	6	
Latakharak	3600	Sholu*-Bhojpatra* dominant habitat	1	1	1	3	Soil, rocks, wood	10	9	+
Latakharak	3700	Angod*-Sholu*-Bhojpatra* dominant habitat	1	1	2	2	Soil, rocks, wood	10	5	
Latakharak	3700	Angod*-dominated habitat	1	1	2	2	Soil, rocks, wood	11	11	+
Latakharak	3700	Takkar*-dominated habitat	2	2	2	2	Soil, rocks, wood	5	5	
Dhonidhar	3800	Alpine pasture	3	3	1	2	Soil, rocks	19	13	+
Jhandidhar	4000	Alpine pasture with morains	3	3	3	2	Soil, rocks	20	11	
Dharansi-Dhoni	4200	Morain-rich alpine pasture	3	3	1	2	Soil, rocks	6	4	
Chokhundadha	4500	Morain-rich alpine pasture	3	3	2	2	Soil, rocks	14	8	

* = Local name.

Botanical names: Kail = *Pinus wallichiana*; Raga = *Abies* sp.; Thuner = *Taxus buccata*; Bhojpatra = *Betula utilis*; Angod = *Rhododendron campanulatum*; Takkar = *Rhododendron anthopogon*; Sholu = *Sorbus foliolosa*.

+ indicates significantly high species richness at 1% level of significance.

1, 2, 3 represent low, moderate and high levels of mesohabitat conditions ranked on ordinal scaling.

Abbreviations: Sn = Exposure to sun; Wn = Exposure to wind; Slp = Habitat slope; Hm = Humidity.

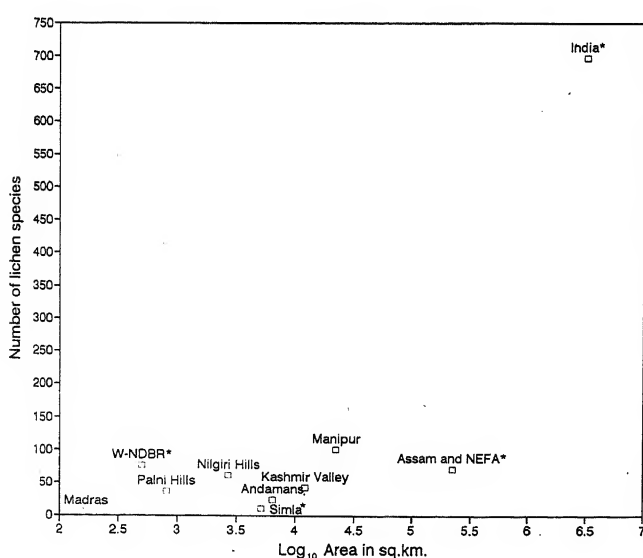


Figure 2. Levels of lichen species richness in the study area (W-NDBR*) with 8 other investigations in different parts of India. *, indicates macrolichen species richness; microlichens have also been included in other studies.

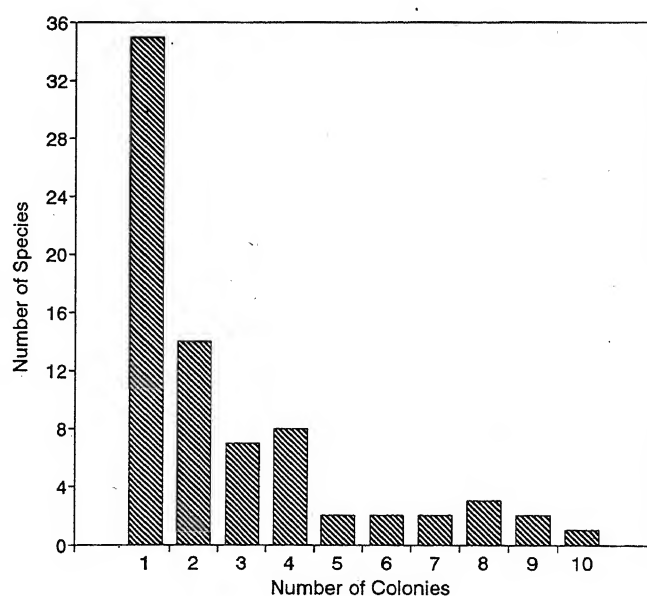


Figure 3. Distribution of relative abundance of 76 species of macrolichens making up a total of 204 colonies over 8000 sq m sampled.

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Table 2. Occurrence of macrolichens with respect to 41 microhabitats

Major substrates	Finer divisions of the substrates (Microhabitats)	No. of transects in which present	No. of colonies encountered	No. of species
Rock	Rock	11	48	16
Soil	Humus-rich soil	5	11	7
	Humus-rich soil on rock	2	4	4
	Humus-rich soil on dead wood log of <i>Rhododendron campanulatum</i>	1	1	1
	Black soil	2	10	5
	Black soil on rock	5	12	9
	Sandy soil on rock	2	5	5
	Red soil	5	2	2
	Moss bed on black soil on rock	2	8	7
	Moss bed on black soil	2	2	2
Wood	Tree trunk bark of <i>Abies pindrow</i>	2	15	14
	Tree trunk bark of <i>Cedrus deodara</i>	1	1	1
	Tree trunk bark of <i>Cupressus torulosa</i>	1	1	1
	Tree trunk bark of <i>Pinus wallichiana</i>	1	5	4
	Tree trunk bark of <i>Taxus buccata</i>	1	1	1
	Tree trunk bark of <i>Betula utilis</i>	4	7	6
	Tree trunk bark of <i>Rhododendron campanulatum</i>	1	2	2
	Standing dead tree trunk bark of <i>Rhododendron campanulatum</i>	1	1	1
	Tree trunk bark of <i>Sorbus foliolosa</i>	1	1	1
	Lower branches of <i>Abies pindrow</i>	2	6	4
	Lower branches of <i>Cedrus deodara</i>	1	1	1
	Lower branches of <i>Pinus wallichiana</i>	1	6	3
	Lower branches of <i>Taxus buccata</i>	1	1	1
	Lower branches of <i>Betula utilis</i>	2	6	3
	Lower branches of <i>Sorbus foliolosa</i>	3	4	3
	Lower branches of <i>Rosa macrophylla</i>	1	1	1
	Fallen dead branch of <i>Abies pindrow</i>	1	1	1
	Fallen dead branch of <i>Cedrus deodara</i>	1	1	1
	Fallen dead branch of <i>Cupressus torulosa</i>	1	2	2
	Fallen dead branch of <i>Pinus wallichiana</i>	1	3	2
	Fallen dead branch of <i>Betula utilis</i>	3	5	3
	Fallen dead branch of <i>Sorbus foliolosa</i>	1	1	1
	Fallen dead branch of <i>Viburnum cotinifolium</i>	1	1	1
	Fallen dead branch of <i>Populus ciliata</i>	1	1	1
	Fallen dead branch of <i>Salix lindleyana</i>	1	1	1
	Dead wood log of <i>Abies pindrow</i>	2	14	9
	Dead wood log of <i>Taxus buccata</i>	1	3	2
	Dead wood log of <i>Betula utilis</i>	2	5	2
	Dead wood of log of <i>Rhododendron campanulatum</i>	1	2	2
	Standing dead tree trunk bark of <i>Cedrus deodara</i>	1	1	1
	Burnt wood log of <i>Pinus wallichiana</i>	1	1	1

considered as broad niched in this context. These include, *Stereocaulon foliolosum*, *Stereocaulon glareosum*, *Cladonia coccifera*, *Phaeophyscia hispidula*, *Cladonia coniocraea*, *Ramalina pollinaria*, *Menegazzia terebrata*, *Ramalina roesleri* and *Cladonia rangiformis*.

Species diversity

Table 2 provides details of the 16 sites in terms of macrohabitat type, altitude, mesohabitats and major substrates. The species richness or α -diversity of these sites was compared through rarefaction¹⁹. On this basis we can assign significantly high levels of species richness at 1% level to 5 sites, viz. Belta at 2700 m, Dibrugghetta at 3400 m, Latakharak at 3600 m and 3700 m and Dhoni-dhar at 3800 m. Notably enough, four of these species-

rich sites harbour all the three major types of substrates.

Species turnover or β -diversity defined as unshared species as a proportion of total species at any two different sites is another component of species diversity²⁰. This is equivalent to 1-Jaccard co-efficient of similarity employed in the complete linkage dendrogram²¹ of Figure 5. It is evident that turnover increases with altitudinal difference. Although microhabitats and mesohabitat conditions at the lowest altitudinal site were similar with alpine meadows, this site separates out because of a distinctive species composition. This may be attributed to the substantial altitudinal difference coupled to human disturbance and air pollution on the road side. The three transects at the same altitude of 3700 m varied in species composition, presumably because of differences in macro-habitat and mesohabitat conditions (Table 1).

Table 3. Macrolichen species, with abbreviated family name in brackets, of western parts of Nanda Devi Biosphere Reserve, arranged in order of decreasing abundance on the three major substrate types, viz. rock, soil and wood

Species {family}	No. of transects in which present	Altitude (× 100 m range)	Rock (1) No. of colonies	Soil (9)		Wood (31)	
				No. of colonies	No. of fine micro-habitats	No. of colonies	No. of fine micro-habitats
<i>Dermatocarpon vellereum</i> Zschacke {Ver}~	3	21-40	9				
<i>Umbilicaria indica</i> Frey {Umb}~	2	37-38	8				
<i>Rhizoplaca chryssoleuca</i> (Smith) Zopf. {Lec.}	1	45	3				
<i>Ramalina himalayensis</i> Ras. {Ram}	1	38	3				
<i>Parmelia sorediosa</i> Almb. {Par}	1	38	1				
<i>Nephroma</i> sp1 {Nep}	1	40	1				
<i>Leptogium furfuraceum</i> (Harm.) Seirk {Col}	1	35	1				
<i>Phaeophyscia constipata</i> (Norrlin in Nyl.) Moberg {Phy}	1	35	1				
<i>Xanthoria elagans</i> (Link.) Th. Fr. {Tel}	1	40	1				
<i>Stereocaulon</i> cf. <i>coniophyllum</i> Lamb. {Ste}	1	38	1				
<i>Stereocaulon foliolosum</i> Nyl.**	5	33-45	7	1	1		
<i>Stereocaulon glareosum</i> (Sav.) Magnusson {Ste}**	5	37-45	4	3	2		
<i>Cladonia coccifera</i> (L.) Wild {Cla}**	3	37-40	1	5	2		
<i>Phaeophyscia hispidula</i> (Ach.) Essl. {Phy}**	4	33-38	2	2	2		
<i>Thamnolia vermicularis</i> (Swartz) Ach. {Sip}	4	35-40		8	5		
<i>Cladonia pyxidata</i> (L.) Hoffm. {Cla}	4	33-45		5	4		
<i>Cladonia ramulosa</i> (With.) Laudon {Cla}	4	34-40		4	3		
<i>Cladonia fimbriata</i> (L.) Fr. {Cla}	3	28-37		3	2		
<i>Peltigera venosa</i> (L.) Hoffm. {Pel}	1	35		2	2		
<i>Peltigera polydactyla</i> (Necker) Hoffm. {Pel}	2	25-36		2	2		
<i>Cetraria everniella</i> (Nyl.) Krempelh. {Par}	2	42-45		2	2		
<i>Cladonia furcata</i> (Huds.) Schrader {Cla}	2	37		2	1		
<i>Cetraria islandica</i> (L.) Ach. {Par}	1	45		1	1		
<i>Heterodermia dissecta</i> var. <i>koyana</i> Kurok. {Phy}	1	33		1	1		
<i>Cladonia rangiferina</i> (L.) Wigg. {Cla}	1	40		1	1		
<i>Cladonia squamosa</i> (Scop.) Hoffm. {Cla}	1	40		1	1		
<i>Peltigera praetextata</i> (Florke ex Sommerf.) Zopt. {Pel}	1	38		1	1		
<i>Cladonia crispata</i> (Ach.) Flotow {Cla}	1	38		1	1		
<i>Cladonia coniocraea</i> (Florke) Sprengel {Cla}**	6	27-37		3	3	7	2
<i>Ramalina pollinaria</i> (Westr.) Ach. {Ram}**	2	33-34	3			1	1
<i>Menegazzia terebrata</i> (Hoffm.) Massal. {Par}**	2	27-28	2			2	1
<i>Ramalina roesleri</i> (Hochst. in Schaerer) Hue {Ram}*	2	27-34		1	1	3	2
<i>Cladonia rangiformis</i> Hoffm. {Cla}**	2	27-40		3	1	1	1
<i>Cladonia chlorophaea</i> (Florke) Sprengel {Cla}	2	37-38		1	1	1	1
<i>Heterodermia speciosa</i> (Wulfen) Trevisan {Phy}	2	27-33		1	1	1	1
<i>Usnea longissima</i> Ach. {Usn}	5	27-37				9	5
<i>Ramalina sinensis</i> Jatta {Ram}	4	25-37				7	4
<i>Lobaria retigera</i> (Bory) Trevisan {Lob}	3	27-34				6	4
<i>Parmelia cirrhata</i> Fr. {Par}*	3	25-28				5	5
<i>Cetraria laureri</i> Krempelh. {Par}	2	25-27				4	3
<i>Parmelia flaventior</i> Stirton {Par}	3	25-34				4	4
<i>Heterodermia leucomela</i> (L.) Poelt {Phy}	2	25-27				3	2
<i>Parmelia nepalensis</i> Taylor {Par}	3	27-36				3	3
<i>Usnea subfloridana</i> Stirton {Usn}	1	35				3	1
<i>Usnea subordida</i> Striton {Usn}	1	25				2	1
<i>Pseudocyphellaria intricata</i> (Delise) Vainio {Sti}	2	27-28				2	1
<i>Usnea orientalis</i> Mot. {Usn}	1	25				2	2
<i>Ramalina taitensis</i> Nyl. {Ram}	1	25				2	1
<i>Sticta praetextata</i> (Ras.) Awasthi in M. Josh & Awasthi {Sti}	2	28-34				2	2
<i>Parmelia praesorediosa</i> Nyl. {Par}	2	34-36				2	2
<i>Cetraria pallescens</i> Schaerer in Moritzi {Par}	2	25-36				2	2
<i>Ramalina</i> sp1 of G. Pant {Ram}	2	25-27				2	2
<i>Parmelia mussooriensis</i> Awasthi {Par}	1	27				1	1
<i>Lobaria isidiosa</i> (Miill. Arg.) Vainio {Lob}	1	37				1	1
<i>Sticta nylanderiana</i> Zahibr. {Sti}	1	34				1	1
<i>Cetraria pinastri</i> (Scop.) Gray {Par}	1	37				1	1
<i>Peltigera</i> sp1 {Pel}	1	27				1	1
<i>Parmelia infumata</i> Nyl. {Par}	1	28				1	1
<i>Heterodermia diademata</i> (Taylor) Awasthi {Phy}	1	27				1	1
<i>Parmelia exasperatula</i> Nyl. {Par}	1	27				1	1
<i>Cetrelia braunsiana</i> (Miill Arg.) Culb. & Culb. {Par}	1	27				1	1
<i>Parmelia xantholepis</i> Mont & Bosch in Jungh. {Par}	1	34				1	1
<i>Parmelia crenata</i> Kurok. in Hale & Kurok. {Par}	1	34				1	1

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Table 3. (Contd)

Species {family}	No. of transects in which present	Altitude (× 100 m range)	Rock (1) No. of colonies	Soil (9)		Wood (31)	
				No. of colonies	No. of fine micro-habitats	No. of colonies	No. of fine micro-habitats
<i>Cladonia submultiformis</i> Asah. {Cla}	1	36				1	1
<i>Cladonia cornuta</i> (L.) Hoffm. {Cla}	1	37				1	1
<i>Usnea baileyi</i> (Stirton) Zahlbr. {Usn}	1	34				1	1
<i>Parmelia austrosinensis</i> Zahlbr. {Par}	1	37				1	1
<i>Lobaria pseudopulmonaria</i> Gyelnik {Lob}	1	37				1	1
<i>Evernia mesomorpha</i> Nyl. {Usn}	1	27				1	1
<i>Usnea</i> sp1 {Usn}	1	35				1	1
<i>Cetrelia olivetorum</i> (Nyl.) Culb. & Culb. {Par}	1	34				1	1
<i>Heterodermia isidiophora</i> (Vainio) Awasthi {Phy}	1	34				1	1
<i>Candelaria concolor</i> (Dicks) B. Stein {Can}	1	37				1	1
<i>Usnea</i> sp2 {Usn}	1	25				1	1
<i>Usnea rubicunda</i> Stirton {Usn}	1	27				1	1
<i>Hypogymnia vittata</i> (Ach.) Parr. {Hyp}	1	37				1	1

‘+’ indicates significantly broad and ‘-’ indicates narrow niche with respect to fine microhabitat preference.

‘++’ indicates broad niched with respect to their occurrence on the major substrates.

Full names of families: {Can}, Candelariaceae; {Cla}, Cladoniaceae; {Col}, Collembataceae; {Hyp}, Hypogymniaceae; {Lec}, Lecanoraceae; {Lob}, Lobariaceae; {Nep}, Nephromataceae; {Par}, Parmeliaceae; {Pel}, Peltigeraceae; {Phy}, Physciaceae; {Ram}, Ramalinaceae; {Sip}, Siphulaceae; {Ste}, Steriocalaceae; {Sti}, Stictaceae; {Tel}, Teloschistaceae; {Umb}, Umbilicariaceae; {Usn}, Usneaceae; {Ver}, Verrucariaceae.

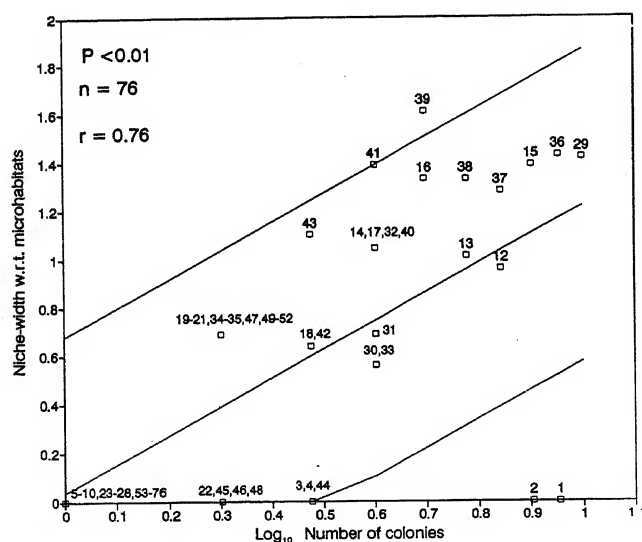


Figure 4. Relationship between the number of colonies encountered and the niche-width of the species with respect to microhabitat usage. The graph also shows the fitted regression line and lines representing confidence intervals at $P < 0.01$. The numbers correspond to the order in which species are listed in Table 3, indicating that *Parmelia cirrhata* has significantly broadened niche and *Umbilicaria indica* and *Dermatocarpon vellereum* have significantly narrow niche-width.

Niche-overlap

Niche-overlap with respect to microhabitat usage was computed based on Pianka's measure of niche-overlap²². Figure 6 based on complete linkage analysis²¹ depicts the clustering of 20 species occurring in four or more colonies with respect to their preference for the 41

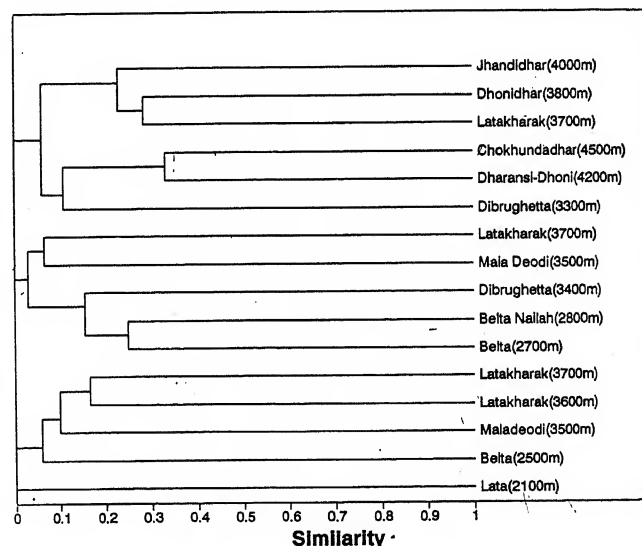


Figure 5. Complete linkage dendrogram of 16 sites based on Jaccard's co-efficient of similarity with respect to composition of species.

microhabitats. The species tend to cluster with respect to the major substrates which are evidently critical in determining the distribution of macrolichen species.

Microhabitat preference

Although woody microhabitats were restricted to 10 out of 16 transects, a majority of the species were found to be favouring wood. Figure 7 is a Venn diagram of microhabitat preference of 20 more abundant as well as 76 total species. These bring out the importance of woody microhabitats in promoting lichen species diver-

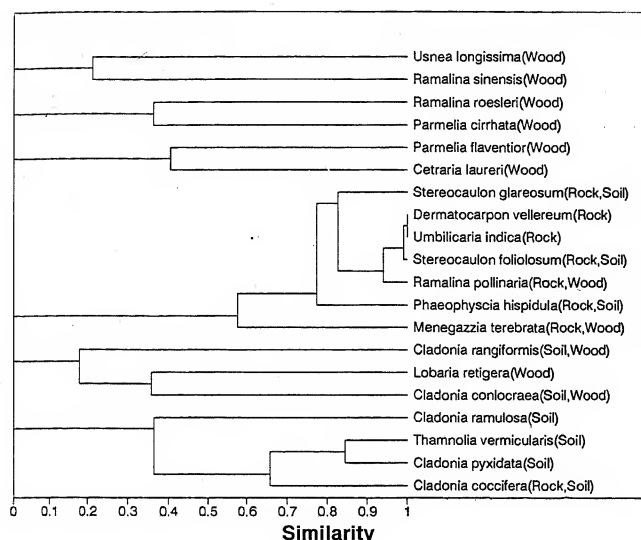
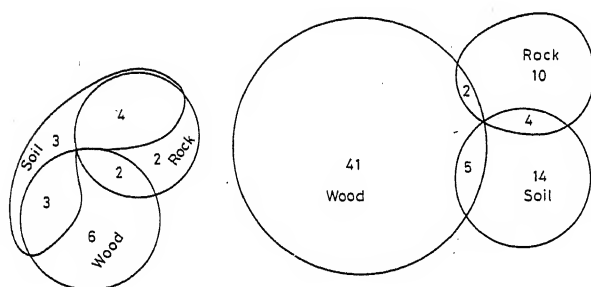


Figure 6. Complete linkage dendrogram for 20 species occurring in 4 or more colonies based on Pianka's measure of niche-overlap with respect to microhabitat usage.



More abundant 20 Species

Total 76 Species

Figure 7. Venn diagram of distribution of a total 76 and of 20 more abundant macrolichen species on the three major substrates.

sity. However there are a number of species confined to soil and rocks as well.

Conservation implications

Deforestation, excessive fuelwood collection and fire²³, all adversely affecting wood loving species of lichens appear to be major threats to the rich macrolichen flora of the Himalayas. Although firm evidence is lacking, it is also possible that the ban on summer grazing in the alpine meadows of NDBR may be adversely affecting macrolichen diversity, since this cessation of grazing has led to spread of weedy species like *Rumex nepalensis*

and *Phlomis bracteata* and shrinkage of soil microhabitats of the lichens. The rich macrolichen flora of Himalayas could play an important economic role as well. Species of *Ramalina*, *Parmelia*, *Usnea* and *Evernia* are a source of essential oils widely used in health care and perfumery industry⁷. Lichen thalli, a rich source of nitrogen²⁴, has promise as a source of organic manure, although today it is being wasted along with fire wood.

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Transformation of *Streptococcus* sp. by cholera toxin gene

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Calcium chloride-treated cells prepared from *Streptococcus* sp., a curd-forming bacterium isolated from whey, were transformed by pCVD15 DNA containing the cholera toxin gene. The transformants conferred resistance to ampicillin and chloramphenicol. Transformation efficiency was slightly higher for the competent cells than for the incompetent cells. The *Streptococcus* cells were found to be naturally competent.

CHOLERA is a life-threatening infectious disease caused by *Vibrio cholerae* colonizing the small bowel and secreting enterotoxin – cholera toxin – that leads to diarrhoeal disease characterized by profuse water loss. Cholera toxin consists of a single A subunit (27 kDa) and 5-B-subunit (11.6 kDa)¹ which binds to the GM ganglioside receptor of intestinal mucosal cells^{2,3}. This binding can be prevented by antibody against B-subunit^{4,5}. Thus B-subunit of cholera toxin gives protection against *V. cholerae* when antibody is located in the lumen of the intestine⁶. Circulating antibody has been proved to be ineffective in giving protection. However, protection by B-subunit is of short duration (few months). Attempts to administer vaccine orally has been unsuccessful because of lack of colonization ability of the strain⁷. For effective use of B-subunit as vaccine the gene has been cloned in *E. coli*⁸, though the extraction and purification costs are very high. Recently trial is being made to inactivate the cholera toxin gene by modifying amino acid residues at positions 7 and 112 of cholera A subunit for use as oral vaccine by exchanging this modified toxin gene to the wild type strain⁹.

An effective way has been devised by Haq *et al.*¹⁰ by making transgenic plant as expression and delivery system for oral vaccine. Plants have expressed the B-subunit gene and feeding of such transgenic potatoes to mice induced protection to treated mice against cholera.

In an alternative way we have devised a system for delivery of oral vaccine against diarrhoeal disease through gram-positive lactic acid curd-forming bacterium *Streptococcus* sp. isolated from whey. Since the nontoxicogenic *V. cholerae* with only B-subunit gene incorporated into its chromosome does not colonize permanently in the intestine⁷, gram-positive bacteria can be tested for this purpose. Lactic acid curd-forming bacteria are safe to eat through curd. Moreover, no extraction and puri-

fication of vaccine is needed. Frequent consumption of curd by this recombinant cultures will maintain the availability of vaccine in the lumen. Also the vaccine can be made at home by making curd, and in our laboratory it has been shown that curd, using this culture, can be made within 4 h. No data, however, is available whether this bacterium does colonize in the intestine.

In this study, *Streptococcus* sp. was transformed with recombinant plasmid pCVD15⁷ containing the entire cholera toxin gene⁷. The transformants conferred resistance to ampicillin and chloramphenicol and had the ability to make curd. This result suggests that both the antibiotic genes have been expressed in *Streptococcus* sp. Since this plasmid does not replicate in gram-positive bacteria, it may be assumed that the plasmid has integrated into bacterial chromosome. Plasmid preparation from the transformant colonies in the presence of antibiotics was done and no plasmid of 18 kb (pCVD15) was found. The number of transformants (2×10^4 μ g CsCl purified pDNA) indicates that this strain shows high frequency of gene transfer and thus can be manipulated genetically.

The working strain was found to be naturally competent. Transformation frequency of *Streptococcus* cells grown in TGE medium¹¹ was 1.5×10^4 (Table 1) whereas it is 2×10^4 when cells were made competent (CaCl method)¹². This observation was verified by transforming both competent and incompetent cells of *Streptococcus* with pUC19 DNA containing ampicillin resistance gene. The transformation frequency was higher for pUC19 DNA than for pCVD15 (Table 1). The mechanism of genetic exchange was proved to be dependent upon uptake of free DNA since no transformants were observed when 20 μ g of pancreatic DNase (in 5 μ l of 0.02 M MgCl, 0.02 M maleate, pH 6.5) was added to transforming DNA one hour before transformation. The transformants were destroyed after completion of experiments as safety measure.

Work is in progress to find out whether the toxin

Table 1. Transformants (chromosomal integrations) efficiency in competent and incompetent cells of *Streptococcus* sp.

Plasmid used	Transformants (μ g DNA)
Competent cells	
a) pCVD15, 5 ng	2×10^4
b) pCVD15, 10 ng	1.8×10^4
c) pUC19, 5 ng	2×10^5
d) pUC19, 10 ng	2.03×10^5
Incompetent cells	
a) pCVD15, 5 ng	1.5×10^4
b) pUC19, 5 ng	8.4×10^4

gene has integrated into bacterial chromosome or it has expressed.

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Occurrence of a 'mariner' element in the silkworm *Bombyx mori*

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Occurrence of mariner element in *Bombyx mori* is reported here for the first time using degenerate primers in polymerase chain reaction (PCR) technique. The PCR product (Bm.MAR1) has been cloned and sequenced; it is 457 bp in length. DNA and peptide sequences of *Bombyx* mariner show a high degree of similarity with the mariner of the predatory mite, *Metaseiulus occidentalis* and low degree of similarity with the lepidopteran *Hyalophora cecropia*. Since no transcripts could be identified for the mariner of *B. mori* in northern hybridization, it appears to be a transcriptionally defective element.

SINCE McClintock¹ discovered mobile genetic element in maize, presence of such transposable elements has

been reported from a number of other eukaryotic genomes². A new transposable element 'mariner' has been identified and cloned from an unstable white mutant of *Drosophila mauritiana*, a sibling species of *D. melanogaster*³. The original mariner element is 1286 bp in length having 28 bp inverted repeats and an open reading frame (ORF) coding for a polypeptide of 345 amino acids. It is functional for germline transformation of *Drosophila*³. Lidholm *et al.*⁵ identified a mariner element in *Hyalophora cecropia* and showed about 40% DNA sequence identity with *Drosophila* mariner element. Using degenerate PCR primers, Robertson⁶ has shown the widespread occurrence of mariner elements in insects (17% among 400 species screened). Using the same PCR primers, Jeyaprakash and Hoy⁷ cloned a mariner element from a predatory mite *Metaseiulus occidentalis* and showed the presence of functional open reading frame. They constructed a transformation vector containing the mariner and made stable transformation of the predatory mite. Despite the fact that more than 400 species have been screened, the presence of mariner elements in the silkworm *Bombyx mori* has not been detected. This paper is the first report on the occurrence of mariner elements in *Bombyx mori*.

Bombyx mori strains (NB₄D₂, NB₁₈, KA, NB₇) were obtained from the Central Sericultural Research and Training Institute, Mysore. The genomic DNA isolation (from larval fatbody cells) was performed as described by Sambrook *et al.*⁸

The PCR was carried out using degenerate primers [MAR124F-5'-TGGGTNCCNCAYGARYT (17-mer) and MAR276R-5'-GGNGCNARRTCNGGNSWRTA (20-mer)⁶]. The conditions for PCR were: *Taq* polymerase buffer (1x), 2.5 mM MgCl₂, 150 µM dNTPs, 800 nM each primer, 0.25 units *Taq* polymerase, and 1-100 ng genomic DNA in a 25 µl total reaction volume⁹. For 35 cycles, amplification conditions were: denaturation at 94°C for 30 sec, annealing at 48°C for 30 sec and extension at 74°C for 1 min in Perkin-Elmer Thermol cycler. An aliquot from each reaction (10 µl) was electrophoresed on a 2% TBE gel. The amplified PCR product was purified by electro-elution and cloned into 'T' tailed plasmid. The clone was termed as pBm.MAR1.

The recombinant plasmid containing pBm.MAR1 was isolated and purified following standard methods⁸. The recombinant plasmid was transformed into *E. coli* strain DH5α; the plasmid DNA was isolated, purified and sequenced by conventional dideoxy method in an automated sequencer¹⁰. The sequence of *B. mori* mariner element was translated and compared with the translated sequence of mariner element of other species using the software 'GCG' (Genetic Computer Group, Wisconsin-Madison, USA)¹¹. Mariner sequences of other species were recovered from 'EMBL Gene bank' for comparison.

Using degenerate primers (MAR124F and MAR276R)

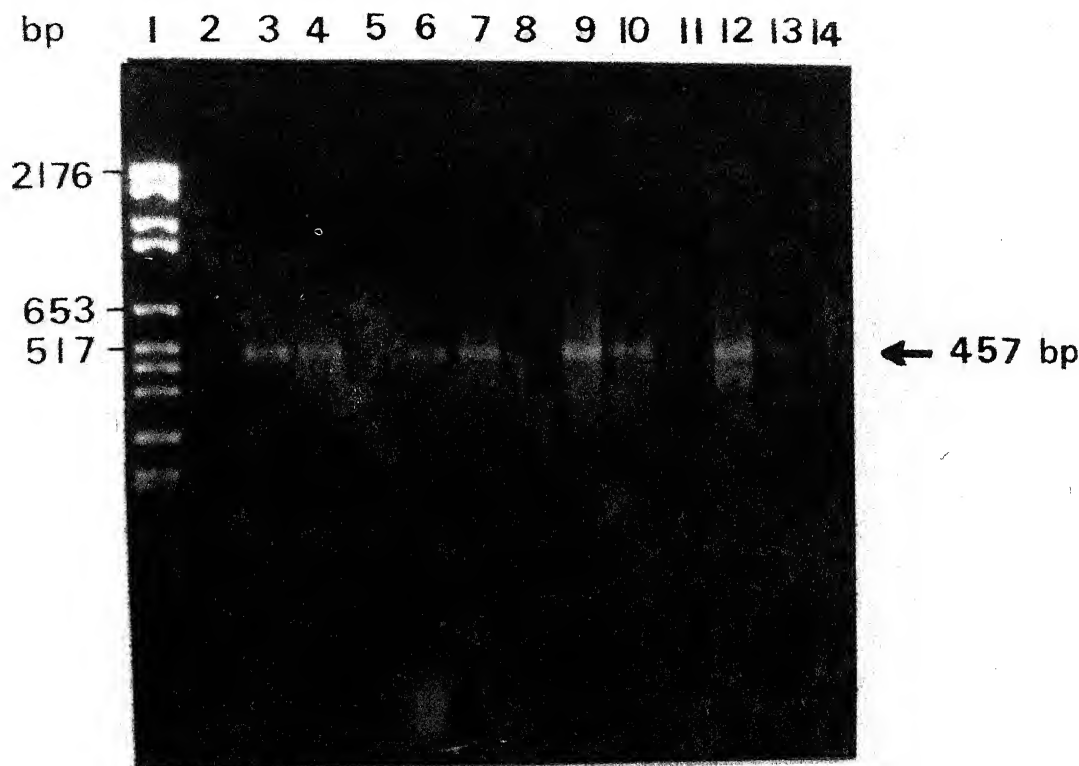


Figure 1. PCR amplification of mariner element from four different strains of *Bombyx mori* using degenerate primers (MAR 124F and MAR 276R). The samples in the lanes are: 1) λ marker; 2) No DNA control; 3) 100 ng, 4) 10 ng and 5) 1 ng of NB₄D₂ genomic DNA used for PCR; 6) 100 ng, 7) 10 ng and 8) 1 ng of NB₁₈ genomic DNA used for PCR; 9) 100 ng, 10) 10 ng and 11) 1 ng of KA genomic DNA used for PCR; 12) 100 ng, 13) 10 ng and 14) 1 ng of NB₇ genomic DNA used for PCR.

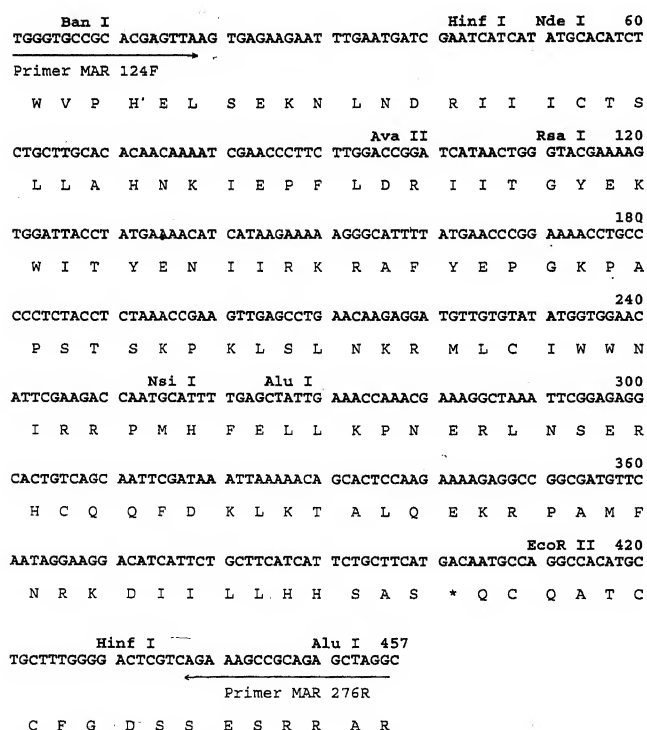


Figure 2. The PCR amplified *B. mori* mariner (Bm.MAR1) sequence was cloned and sequenced. The restriction map and amino acid sequence of Bm.MAR1 are shown.

PCR amplification of genomic DNA from *B. mori* strains resulted in the production of a single fragment approximately 450 bp. The PCR products obtained in all the four strains were identical in size (Figure 1). The PCR product was equal in size to the mariner product amplified in *D. mauritiana* using the same primers⁶. The PCR product from *B. mori* represents internal sequence of the mariner and the purified product was cloned. Six clones were obtained from the PCR product amplified from *B. mori* strain NB₇, and all the clones showed identical size on agarose gel fractionation. One of the clones was completely sequenced and it contained 457 base pairs (Figure 2). The possible existence of an ORF was tested in all the three frames. There were a number of stop codons in the second and third frames, while there was one stop codon in the first reading frame at 399 bp. The 457 bp pBm.MAR1 was compared with PCR-amplified clones (using the same primers) of mariner from *Hyalophora cecropia*, *Anopheles gambiae*, *Haemaphysa irritans*, *Metaseiulus occidentalis* (using 'GAP' software of GCG) and the data is presented in Table 1. The mariner sequences of the above species were translated into amino acid sequences and aligned using Clustal V multiple sequence alignment program of GCG software (Figure 3). Peptide similarity and identity of the mariner from these species were compared. *M.*

<i>B.mori</i>	SEKNLNDRIII---CTSLAHNKIEPFLDRIITGYEKWITYENIIRKRA---FYEPGKPAPSTSKPKLSLNKRMLCIWW
<i>H.cecropia</i>	SESNLQTRVDC---YVTLNLRHNNERKMYDNKRGSLQWL-----NPGDPAKSCPKR*LTQKKLLVSVLV
<i>M.occidentalis</i>	SERQKEVRLTV---CRELLSRYKNKSFLYRIITSDEKWIYYDNPGRKRS---WVSPGEPAPKSVRRNRFGKKTMLCVWW
<i>D.mauritiana</i>	NERQMERRKNT---CEILLSRYKRKSLHRIITGDEKWIFFVNPGRKRS---YVDPGQPATSTARPNRFGKKTMLCVWW
<i>A.gambiae</i>	TFDQKHQRVDDSERCLQLLTRNTPE-FLRRYVTMDQTLWHHYTPESNRKSAQWTATDEPAPKRGKTQKSAGKVIASVFW
<i>H.irritans</i>	TFDQKQQRVDDSERCLQLLTRNTPE-FFRRYVTMDETLWHHYTPEFDQQAETATGEPSPKRGKTQKSAGKVMASVFW
	. . R . . LL . . W . . . P . . K . .

<i>B.mori</i>	NIRRPMPHFELL-KPNERLNSERHCQQQFDKLTALQEKRPAMFNKDIILLHHSAS*QQATCCFGDS
<i>H.cecropia</i>	D*RRCHSLQ-LSKMWPNDYSRYLLSATASHEGRTSC*TSEIGQSL*VTAASRQRKTHC*TNNH*VK
<i>M.occidentalis</i>	DQRGVIYHELL-KPGETVDTARYQQQLIDLNRVKEKRPNWDQVRN-ASF CSTTTL SVTPSRRRRL
<i>D.mauritiana</i>	DQSGVIYELL-KPGETVNTARYQQQLINLRALQKRPEYQKRQHRVIFLHDNAPSHTARAVRDTL
<i>A.gambiae</i>	DAHGIIFIDYLEKGKTINSDYMALSERFKVEIAA-KRPHMKKKK--VLFHQDNAPCHKSLRTMAKI
<i>H.irritans</i>	NAHGIIIFIDYLEKEKTINSDYMAL*RLKVEIAA-KWPHMKKKKVLFDQ--DNAPCHKSVRTMAKI
	L K . . K . . DNAP

Figure 3. Alignment of translations of mariner PCR fragments from *D. mauritiana*, *H. cecropia*, *A. gambiae*, *H. irritans*, *M. occidentalis* and *B. mori*. They were translated using 'GCG' software; the '-' symbol indicates frameshift to maintain an aligned reading frame and the asterisk indicates stop codon. The translations were aligned using Clustal V multiple sequence alignment program with final manual alignment.

Table 1. Comparison of Bm.MAR1 sequence with mariner elements of other species. The mariner sequences were retrieved from 'EMBL' data bank and compared using 'GAP' program of 'GCG' software

Species	DNA sequence identity (%)	Peptide similarity (%)	Peptide identity (%)
<i>D. mauritiana</i>	57	52	39
<i>H. cecropia</i>	47	44	25
<i>A. gambiae</i>	39	46	24
<i>H. irritans</i>	41	48	27
<i>M. occidentalis</i>	49	57	41

occidentalis showed maximum peptide similarity (57%) with *B. mori* mariner sequence, and minimum similarity was noted with *H. cecropia* (44%). In peptide identity, the maximum similarity was observed with *M. occidentalis* and minimum with *A. gambiae*. Though *B. mori* and *H. cecropia* belong to the same order Lepidoptera, their mariner elements are diversified. The mariner element of *B. mori* appears to be close to *M. occidentalis* or *D. mauritiana*.

The northern blot of total RNA from *B. mori* was probed with Bm.MAR1. No detectable signal was obtained, indicating the absence of mariner specific mRNA population. Since there was a break in the coding sequence of *B. mori* mariner, it was found to be a defective mariner. Possible use of the mariner for the construction of vector for a stable germline transformation of *B. mori* needs to be confirmed only after cloning and sequencing of a number of mariner elements from more silkworm strains (both mulberry and non-mulberry

silkworms). This is the first report on *B. mori* mariner and cloning of full length mariner from other strains is under progress.

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Methane production by Indian ruminant livestock

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Methane production estimates for Indian ruminants by various outside agencies were much higher^{1,2} because the estimates were based on theoretical calculation without any actual experimentation on Indian ruminant livestock. The total methane contribution to greenhouse pool by Indian ruminants was 9.02 Tg/year based on methane production per kg digestible dry matter of feed available in different parts of the country and determined at National Dairy Research Institute, Karnal. Similarly, the methane production per kg milk produced in India was also much lower than reported earlier³.

CARBON DIOXIDE, methane, chlorofluorocarbons (CFCs), nitrous oxide and other gases like sulphur hexafluoride etc. are the main greenhouse gases accumulating in the atmosphere due to the process of industrialization. This is causing depletion of ozone layer and global warming. It has been projected that if the present state of industrialization, dairy farming, growing of paddy, etc. con-

tinue, the concentration of carbon dioxide in atmosphere will double⁴ by 2055 AD and concentration of greenhouse gases from all sources (carbon dioxide, methane, nitrous oxide, CFC, etc.) will double⁵ by 2035 AD. Methane emission in atmosphere as a greenhouse gas has been estimated to be 255 million tonnes per year², although, Reid⁶ estimated 423 million tonnes per year. Methane contribution to greenhouse gas pool is only 18%, however, its production by ruminants is one of the major global issues because of its relative effectiveness in terms of global warming potential and life in atmosphere. One kg methane's relative effectiveness in terms of global warming potential is equal to 40 kg carbon dioxide. According to an estimate², Indian contribution of methane from all sources is 12.1% of total world methane production and out of that rice field and ruminants contribute 27.3 and 13.2%, respectively, of total Indian methane production. Various methods are suggested for reducing the methane production by ruminants, however, control of methanogenesis by manipulation of rumen fermentation through feed additive and feeds is the only feasible and attractive solution at present^{7,8}. Acetate and butyrate are methanogenic and spare hydrogen during their formation, while propionate being the glucogenic VFA utilizes hydrogen. Therefore, molar percentage of acetate, propionate and butyrate in TVFA play an important role

Table 1. Methane production by Indian ruminant livestock (cattle, buffalo, sheep and goat)

Species	Methane production			
	Number ($\times 10^3$)	l/day ($\times 10^6$)	mole/day ($\times 10^8$)	Tg/year
<i>Cattle crossbred</i>				
Male	4278	5.00	0.26	0.154
Female	8513	12.48	0.56	0.322
<i>Cattle indigenous</i>				
Male	94407	112.60	5.00	2.934
Female	88512	87.50	3.90	2.280
Total	195867	218.50	9.72	5.690
<i>Buffalo</i>				
Male	16706	19.10	0.85	0.498
Female	60054	85.80	3.83	2.237
Total	76760	108.90	4.86	2.735
<i>Sheep</i>	44,837	8.00	0.36	0.388
<i>Goat</i>	99405	14.90	0.66	0.388
Total				9.023

Based on:

Animal no.: Tech. Committee Report, Govt. of India, 1993.

Body weight of animal.

Type of feed and intake.

Digestibility of feed.

Methane per kg DDM determined.

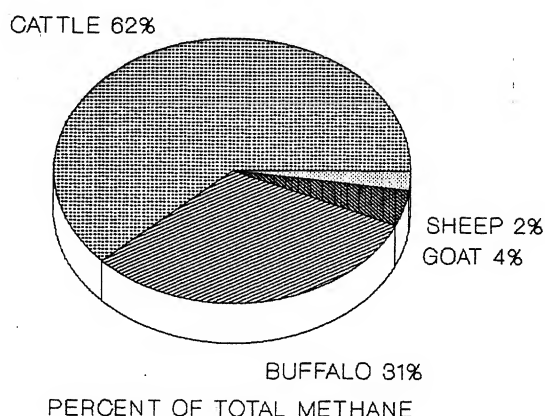


Figure 1. Contribution of methane by different ruminant livestock.

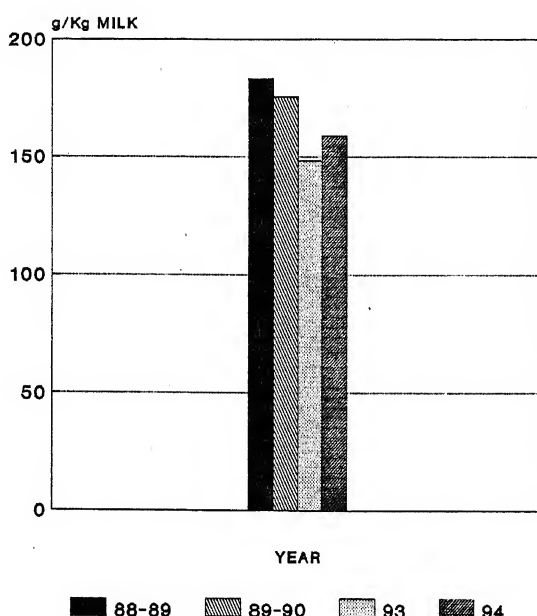


Figure 2. Methane production by Indian ruminant per kg milk produced based on total milk production and population.

in methane production by ruminants and proportion of these in rumen needs to be manipulated.

Total gas production was measured as proposed⁹. At the same time, *in vitro* dry matter digestibility (IVDMD) (single stage) of the samples of feed and fodders along with their combinations as practised in various parts of the country were also estimated for calculating the gas and methane production per unit of digestible dry matter (DDM). Percentage of methane in gas was estimated by absorbing carbon dioxide in 1 N sodium hydroxide, and making necessary correction for some other minor gases present in ruminal gas pool. Methane production per kg digestible dry matter was estimated for different types of ration based on IVDMD and methane produced on fermentation of feed. Thus, methane production based

on per unit feed fermented is comparatively a better method of assessment of methane production. On the basis of methane produced per kg DDM, methane production per day for different categories of animals was calculated taking into account the intake and digestibility of feed by the animals. Total methane production by Indian ruminant livestock has also been calculated.

In rural areas of India, animals are not fed in keeping with their requirements. Most of the animals are underfed during scarcity period of the year. Feeding practices and type of feed differ from region to region. Even the body weight of animals is not the same and varies. Taking into account all these factors total emission of methane from ruminant livestock has been calculated (Table 1). Total emission of methane by cattle is 5.690 Tg/year, with the male contributing 3.088 Tg/year, and female 2.602 Tg/year. Buffaloes contribution of methane to atmosphere is estimated to be 2.735 Tg/year, with male buffaloes contributing only about 18%. Sheep and goat's contribution to total methane pool is 0.210 and 0.388 Tg/year. The per cent share of total methane production was much higher in cattle than the total of buffalo, goat and sheep (Figure 1) because of their numbers, body weight and amount of feed consumed.

Contrary to the amount of methane emission by Indian ruminant livestock observed on the basis of experimental data by the authors, higher amount of methane emission (10.0 and 10.4 Tg) was reported^{1,2} in 1990 on the basis of theoretical calculation. On the other hand, much lower amount of methane emission has been reported¹⁰ which was based on the prediction equation developed in Western countries, feeding grain and high quality hay diet¹¹. This is not applicable to Indian animals because Indian ruminant animals are fed on crop residues-based diets and that too is not enough to meet the nutrient requirement of more than 90% of animals.

In India, the average milk production is not more than three kg per animal per day and despite large numbers of animals majority of them are either non-producers or very low producers. Methane production per kg milk produced in India (total methane produced/total milk production), reported and discussed in many National and International fora, seems to be higher than what is actually produced, (240 g methane per kg milk)³. Methane production per kg milk varies between 148 and 183 g/kg milk (Figure 2) based on milk production and methane production estimates of last 6-7 years. However, it is far less than the reported figure of 240 g/kg.

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Mutants resistant to foliar diseases in groundnut (*Arachis hypogaea* L.)

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Foliar diseases particularly leafspots and rust are the major factors limiting yield and quality in groundnut. Most of the groundnut cultivars in India are highly susceptible to foliar diseases. Fungicidal sprays are effective in controlling these diseases, but the use of disease-resistant cultivars is a better approach. A number of resistant germplasm lines are available but many other undesirable attributes limit their utility as cultivars. Attempts have been made to produce high yielding disease resistant cultivars through hybridization, but the lines developed either had only moderate resistance or retained one or more undesirable features. Mutagenic treatment of Valencia 1 with EMS, resulted in isolation of a large number of foliar disease-resistant mutants. Three mutants, viz. 28-2, 45 and 110 combined high yield potential and early maturity, besides multiple disease resistance and desirable pod and kernel features. These mutants can be widely tested for their commercial release and/or profitably utilized in future breeding programmes.

THE cultivated groundnut (*Arachis hypogaea* L.) is an important oilseed crop and is presently cultivated in an area of 21.17 m ha with a total production of 25.89 m t. In India groundnut occupies 31.3 per cent of the total

cropped area under oilseeds (8.35 m ha) and accounts for 36.1 per cent of total oilseed production (8.85 m t). Like in other developing countries, the average yield in India is around 1000 kg per ha as against the average of 2995 kg per ha realized in USA¹. Many reasons are ascribed to the low productivity in developing countries. Foliar diseases particularly early leafspot (*Cercospora arachidicola* Hori.), late leafspot (*Phaeoisariopsis personata* (Berk and Curt) V. Arx) and rust (*Puccinia arachidis* Speg.) are the major factors limiting yield and quality in groundnut. Each of these diseases is individually capable of causing substantial yield loss, and when leafspots and rust occur together yield losses can go up to 70 per cent². These diseases have an adverse influence on the recovery of pods at harvest, quality of seeds, and haulms. Fowler³ estimated that defoliation of leaflets may begin when six per cent of their leaf area is diseased. Severe infections may cause complete defoliation. However, lesions are not confined to the leaves but may occur also on the stems and pegs, leading to direct deterioration of the developing pods⁴. Because of prolonged wet periods, late leafspot is more predominant in the transitional belt of Karnataka, which accounts for over 50 per cent pod and fodder yield loss⁵. Spanish cultivars are the most popular cultivars in this regions as they mature early and facilitate double cropping under rainfed conditions. But all of them are highly susceptible to the foliar diseases. Though several effective chemicals are available to control these diseases, fungicidal control is not preferred due to the escalation of production cost, especially in the rainfed condition⁶. Cultivation of the resistant/tolerant varieties is the best approach under these circumstances.

Several genotypes resistant to late leafspot and rust have been identified. But most of them belong to the Valencia group and are landraces with a number of undesirable attributes like thick shell, low productivity, late maturity and poor adaptation, making them unsuitable for direct utilization^{7,8}. A number of attempts have been made to produce disease-resistant, productive cultivars through hybridization, but the lines developed either possessed only a moderate level of resistance or retained one or more undesirable features⁹. A number of wild *Arachis* species have shown either highly resistant or immune reaction to these pathogens¹⁰. *A. cardenasii*, a diploid species, was identified to be an excellent source of resistance to late leafspot⁹. Stable interspecific hybrid derivatives belonging to *Virginia* group with high yield and high level of resistance have been developed but they were all late maturing and had low shelling percentages¹¹. Recently two moderately resistant hybrid derivatives, viz. ICGV 86590 and ICGV 87160 were released for commercial cultivation in southern peninsular region of India. They are comparable to popular susceptible cultivar JL 24 in productivity, but suffer from

Table 1. Performance of mutants for resistance to foliar diseases, productivity and pod and kernel features

Genotype	Botanical type	Field disease [#] score (1–9 scale)			Pod yield (g/plant)			Shelling percentage (UPr)	SSI	Days to maturity	Pod features [†]			Kernel [‡] colour
		Leaf- spots	Rust	Cumulative AUDPC	Pr	UPr	Mean (MP)				Beak	Constriction	Reti- culation	
Parents														
DER	*	9	7	2069 ^c	23.1 ^c	11.4 ^g	17.3 ^c	74.1 ^a	6.0	97	S	P	S	LT
VL1	Valencia	9	2	1911 ^d	30.4 ^c	19.3 ^f	24.9 ^d	70.3 ^c	8.6	103	A	A	A	DT
Selected mutants														
28-2	Spanish	5	2	732 ^a	39.7 ^a	36.4 ^a	38.1 ^a	74.0 ^a	1.2	102	A	M	A	DT
45	Spanish	5	3	766 ^a	36.9 ^{ab}	34.2 ^b	35.6 ^b	72.5 ^b	1.3	102	A	M	A	DT
110	Spanish	5	8	842 ^{ab}	32.4 ^c	31.0 ^c	31.7 ^c	72.6 ^b	–0.8	102	A	M	A	DT
Susceptible check														
JL 24	Spanish	9	8	2122 ^c	38.8 ^{ab}	25.9 ^d	32.4 ^c	74.3 ^a	8.5	100	S	S	A	DT
Resistant checks														
GBFDS 272	Virginia	5	2	790 ^{ab}	26.9 ^d	24.0 ^c	25.5 ^d	74.9 ^a	1.9	125	M	M	M	R
PI 259747	Valencia	5	2	993 ^c	24.9 ^d	23.3 ^c	24.1 ^d	74.1 ^a	1.2	118	M	P	P	P
Mean of 23 genotypes		7.4	3.8	1463.30	32.25	25.79	28.99	72.1	3.77	104.7	–	–	–	–
S.E.m.		–	–	21.63	3.34	0.52	0.72	0.44	–	–	–	–	–	–
LSD (5%)		–	–	63.43	2.11	1.52	2.06	1.29	–	–	–	–	–	–

Figure(s) with same subscript(s) do not differ at 5% level of significance.

*Cannot be grouped in any of the four botanical types.

Pr = Fungicide protected and UPr = Unprotected diseased condition.

[†]Pod features: A - Absent, S - Slight, M - Moderate, P - Prominent.

[‡]Kernel colour: LT - Light tan, DT - Dark tan, P - Purple, R - Red.

[#]Field disease score: where 1 = 0%, 2 = 1-5%, 3 = 6-10%, 4 = 11-20%, 5 = 21-30%, 6 = 31-40%, 7 = 41-60%, 8 = 61-80% and 9 = 81-100% damage to foliage.

low shelling outturn, undesirable pod features besides slightly late maturity¹². Hence, there is a strong need to develop and identify new germplasm combining high level of resistance, early maturity, desirable pod and kernel features, besides increased productivity in groundnut. But the information so far available points to the existence of a yield/resistance barrier¹³.

Induced mutagenesis offers an opportunity to artificially create desirable variation. Artificial mutagenesis of Dharwad Early Runner (DER), a stable genotype, with ethyl methane sulphonate (EMS) in our laboratory yielded resistant, early maturing, and erect bunch Valencia mutants (viz. 1, 2, 8, 26, 39, 59 and 83)¹⁴. On subsequent mutagenesis with EMS, mutant-1 (VL 1) yielded secondary mutants (28-2, 45 and 110), which showed a high level of resistance with more desirable attributes¹⁵.

In the present study, 18 induced mutants were evaluated for foliar diseases reaction, productivity, and pod and kernel features along with DER and VL 1 parents and susceptible (JL 24) and resistant (PI 259747 and GBFDS 272) checks under both fungicide protected (Pr) and unprotected (UPr) diseased conditions. The experiment was conducted during the 1995 rainy season in a split plot design with two replications. The main plots consisted of two treatments, viz. (i) As a fungicidal treatment carbendazim (@0.05%), tridemorph (@0.05%) were sprayed to control leafspots and rust respectively at 60, 75 and 90 days after sowing. (ii) As a no fungicide

check treatment water was sprayed at the rate of 500 l/ha. The sub-plots comprised of 23 genotypes. Each genotype, except DER, was grown in a 2 m row with a spacing of 30 cm between rows. The plant to plant distance within a row was 10 cm. In the case of DER line spacing was 60 × 15 cm owing to its procumbent habit. In each replication, all the observations were made on the five randomly selected plants. Each genotype was scored one week before harvest of the crop for leafspots and rust on a 1-9 scale¹⁶. The data collected on leaf area affected due to leaf spots and rust and defoliation at five stages (70, 77, 84, 91 and 98 days after sowing) were used to compute total leaf area lost at different stages. From these data cumulative AUDPC (Area under disease progress curve), an overall indicator of disease resistance was calculated¹⁷. Mean productivity (MP) represented by the average yield under disease protected and non-protected conditions and stress susceptibility index (SSI)¹⁸ were used as criteria for assessing genotypes for diseases tolerance. The genotypes with higher MP and lower SSI were considered as productive and tolerant. After harvesting pods were washed, and dried, pod yield per plant (g) and shelling percentage were determined. Pod (beak, constriction and reticulation) and kernel (colour) features were recorded as per the descriptor published by IBPGR¹⁹.

As indicated by the field disease scores and cumulative AUDPC values, the incidence of leafspots and/or rust was significantly lower in the mutants than the susceptible

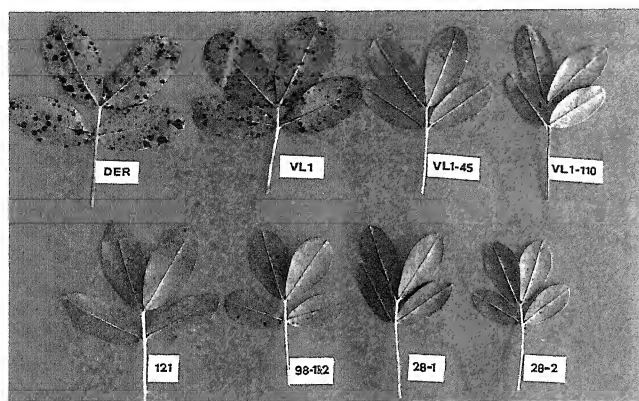


Figure 1. Disease development on parents (DER and VL 1) and selected mutants.

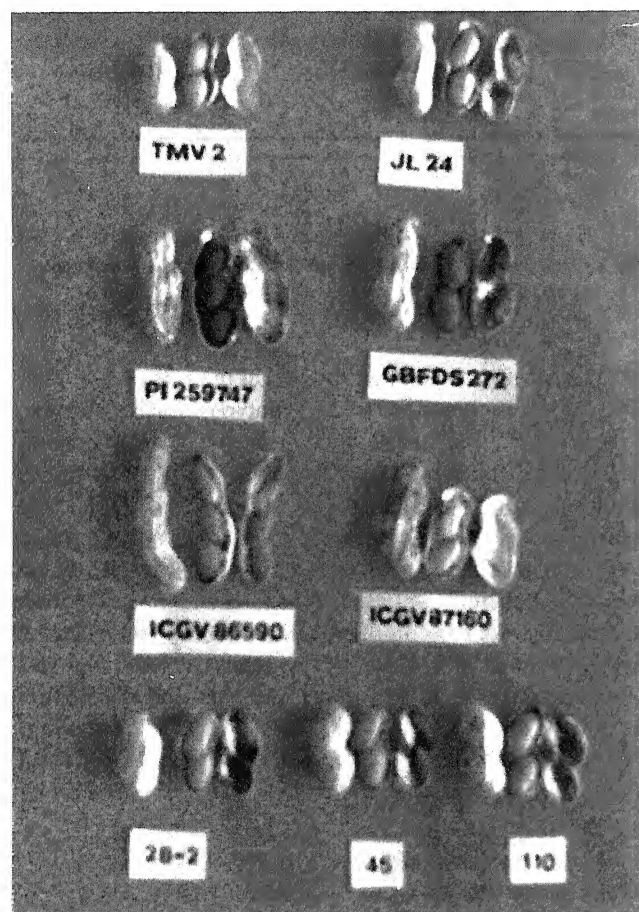


Figure 2. Pod and kernel features of susceptible cultivars (TMV 2 and JL 24), resistant germplasm (PI 259747 and GBFDS 272), resistant cultivars (ICGV 86590 and ICGV 87160) and selected mutants (28-2, 45 and 110).

check and parents (Table 1 and also Figure 1). The resistant Spanish mutants (28-2, 45 and 110) matured

early (103 days) compared to resistant checks. They also had desirable pod and kernel features, viz. smooth pods and tan kernels (Figure 2) and were comparable to susceptible check (JL 24) for yield and shelling outturn. They also recorded lower stress susceptibility index and higher mean productivity (Table 1). These mutants can be widely tested for their commercial release and/or profitably utilized in future breeding programmes.

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Visual evidence of nitrate reductase exudation from plant roots

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To prove the nitrate reductase exudation, roots of 22 plant species were kept in contact with filter sheet impregnated with *N*-(1-naphthyl)ethyl-diamine dihydrochloride, sulphanilamide and potassium nitrate. Nitrate reductase exudation was indicated by the appearance of root impressions in red colour. Nitrate reductase exudation was observed from the roots of 13 plant species even though roots of all species showed its activity.

NITRATE reductase (NR) catalyses the conversion of NO_3 to NO_2 (first step in denitrification process)^{1,2} and its high activity may lead to high N losses from soil^{3,4}. Like other soil enzymes, i.e. urease and phosphatase, microorganisms are known to be the source of its activity in soil². Plants may also effect the enzyme status in soil either by affecting the microbial population in rhizosphere or by directly contributing towards its pool as root exudates as in the case of urease and phosphatase^{5,6}. However, it is not known whether plant contributes directly towards NR activity in soils. Such an information may be useful in devising strategies for reducing NR activity in soil and controlling denitrification losses. Here, we describe a technique to find out whether grasses, crops and trees (Table 1) contribute directly towards NR activity in soil as root exudates.

A sheet of Whatman No. 1 filter paper was spread on a glass plate (60 × 45 cm) in a slanting position and clamped. Distilled water (100 ml) was gradually poured from the upper end of the sheet three to four times for washing. The sheet was then allowed to dry at room temperature. Subsequently, a mixture of *N*-(1 naphthyl) ethyl diamine dihydrochloride (NED, 500 ppm aqueous solution) and sulphanilamide (SA, 1% w/v aqueous solution), mixed in equal proportion was sprayed on the sheet through a chromatographic sprayer and was then dried in dark. Once again, the sheet was sprayed with potassium nitrate solution (100 ppm $\text{NO}_3\text{-N}$) and dried in dark. Next, the sheet was cut into 25 × 10 cm pieces and fixed on glass plate (20 × 7.5 cm) by folding and sticking the excess portion of the sheet on the reverse side of the plate with a cellophane tape.

Selected plants were irrigated and this facilitated up-rooting of these plants with their roots intact. Subsequently, roots were washed, first under flowing tap water and then with distilled water to remove adhering soil particles. Roots were mildly jerked to remove water droplets. Thereafter, roots were spread on to the glass plate and covered with a similar type of plate. Both

plates were held together by rubber bands and kept in dark for 4 h.

It was hypothesized that if exuded, NR will reduce NO_3 present on filter sheet to NO_2 . NO_2 would then diazotize with NED and SA and give the characteristic red colour⁷. Roots of *Cymopsis tetragonoloba* (var. Maru guar), *Vigna radiata* (var. S-8), *Vigna aconitifolia* (var. Maru moth), *Vigna unguiculata* (var. Pusa aseem), *Cicer arietinum* (var. BCG-9), *Zea mays* (var. Megha), *Triticum aestivum* (var. PBW 175), *Azadirachta indica*, *Albizia lebbek*, *Prosopis juliflora*, *Ficus religiosa*, *Simmondsia chinensis* and *Acacia ampliceps* left perfect impression in red colour on the filter paper (Figure 1). These impressions indicated the exudation of NR from roots.

However, theoretically the impressions would have also formed had NO_2 been exuded from roots. To explore this possibility roots were kept in contact with freshly prepared filter sheet sprayed only with NED and SA and not with KNO_3 . Absence of red impressions would confirm exudation of only NR by ruling out NO_2 exudation. However, we observed red-coloured root impressions in this case also. Though, exudation of NO_2 appears highly unlikely as it is toxic, highly unstable and is rapidly converted to NH_4 in plant⁸, but without discounting the possibility of its exudation, NR exudation cannot be proved. To test this, inhibition of root NR by sodium tungstate was tried⁹. The hypothesis was that

Table 1. Activities of nitrate reductase in roots of different plants

Species	Nitrate reductase ($\mu\text{g NO}_2\text{-N/g root}$ tissue/h)	Root impression
Crops		
<i>Vigna unguiculata</i>	1.00	Yes
<i>Cymopsis tetragonoloba</i>	7.71	Yes
<i>Vigna radiata</i>	2.48	Yes
<i>Vigna aconitifolia</i>	1.31	Yes
<i>Triticum aestivum</i>	5.80	Yes
<i>Zea mays</i>	9.90	Yes
<i>Cicer arietinum</i>	9.90	Yes
<i>Pennisetum glaucum</i>	3.13	No
<i>Sesamum indicum</i>	3.40	No
<i>Ricinus communis</i>	9.89	No
Trees		
<i>Albizia lebbek</i>	1.31	Yes
<i>Acacia ampliceps</i>	1.16	Yes
<i>Ficus religiosa</i>	0.87	Yes
<i>Azadirachta indica</i>	0.74	Yes
<i>Simmondsia chinensis</i>	0.44	Yes
<i>Prosopis juliflora</i>	0.54	Yes
<i>Acacia nilotica</i>	0.64	No
<i>Dichrostachys nutans</i>	0.55	No
<i>Colophospermum mopane</i>	0.65	No
<i>Acacia aneura</i>	0.58	No
Grasses		
<i>Lasiurus indicus</i>	9.45	No
<i>Cenchrus ciliaris</i>	10.39	No



Figure 1. Root impressions showing of nitrate reductase. '+' and '-' indicate exudation and no exudation of NR respectively.

appearance of red-coloured impressions even after NR inhibition would demonstrate NO_2 exudation while lack of it would show NR exudation. For this, plants were irrigated with $100 \mu\text{M}$ solution of sodium tungstate (Na_2WO_4) twelve hours before uprooting. Inhibition of root NR was confirmed by *in vivo* assay¹⁰. Roots irrigated with Na_2WO_4 did not produce red impressions on filter paper impregnated with (i) NED + SA only or (ii) NED + SA + NO_3 . Absence of coloured root impression in (ii) demonstrated complete inhibition of root NR by sodium tungstate because otherwise we would have observed at least a faint impression. These observations led us to conclude that NO_2 is not exuded. Therefore, our earlier observation (roots with uninhibited NR in contact with NED + SA only) of red root impressions on filter sheet suggests the formation of NO_2 on root surface. This is possible only if both NO_3 and NR are exuded. Exudation of NO_3 has also been reported in *Lolium perenne*¹¹. Further, Figure 1 suggests that NR is exuded from the entire root system rather than any specific site.

Root of *Pennisetum glaucum* (var. MH 179), *Sesamum indicum* (var. C 50), *Ricinus communis* (var. Aruna), *Colophospermum mopane*, *Acacia aneura*, *Dichrostachys nutans* and *Acacia nilotica* however, did not exude NR.

Estimation of NR in roots¹⁰ indicated its activity in

all plants (Table 1). However it was surprising that 7 crops out of 10, 6 trees out of 10 and none of the tested grasses showed its exudation. Plants studied belonged to different families but all plants of even one family did not exude NR. Therefore, it appears that exudation or lack of it was clearly a character of individual plant species.

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Minerals and Metals in Ancient India, Vol. 1 **Archaeological Evidence**, by Arun Kumar Biswas (524 + xxix pp); Vol. 2 **Literary Evidence**, by Arun Kumar Biswas and Sulekha Biswas (259 + xvii pp). D. K. Print World (P) Ltd., New Delhi 110 015. 1996. Price: Rs. 3500 (US \$ 225) per set.

The writing of History involves a reconstruction of the past, through a process of research, making use of a variety of source materials. The sources of information may be in the form of written material (inscriptions, travellers' accounts, the literature of the period), material objects and structures (as handed down from the past, or as unearthed through the work of archaeologists), and even traditions (in the form of practices, folklore). The historical researcher draws upon all the available evidence, and exercises his/her reason and judgement to construct a coherent picture of the happenings in the past, consistent with the available evidence, and with the best of objectivity. It is a continuing process, where every painstaking and careful effort is valuable.

The construction of Indian history is beset with many problems. The civilization itself had its beginnings several millennia back, before the appearance of any literature or documentation. Through the ages, a good portion of the material achievements has been destroyed as a consequence of natural calamities and even more, the insensitive and brutal ravages by invaders and aggressions from outside. At the same time, it is so important to reconstruct and recapture the cultural and intellectual heritage of India's past, particularly from the point of view of the younger generation in the country, to give them a sense of legitimate pride and meaningful belonging to the motherland. Fresh discoveries by archaeologists, modern scientific techniques of dating and materials characterization, and the capabilities offered by the computer have all added new dimensions to the study of history.

One of the illustrious and earliest attempts to write a history of Indian science was by the late Acharya Prafulla Chandra Ray, who published his 'monumental work', *the History of Hindu Chemistry*, in two volumes during the period 1902 to 1908. This classic was later republished with additions, in

1956, under the title *History of Chemistry in Ancient and Medieval India* as edited by P. Ray (then Professor of Chemistry at the Indian Association for the Cultivation of Science, Calcutta). The enlarged edition has covered the period from pre-Harappan days (4th millennium BC) till the end of the Moghul period. It has included extensive references to the Sanskrit literature of the Vedic and Ayurvedic period, the literature of alchemy and also a section on metallurgy and working of metals.

There has been a natural and growing interest in research on the history of Indian science, since the time of our political independence (1947) and various initiatives have been taken by the Government and the science academies. Arising out of the recommendations from a symposium on the History of Sciences in South-East Asia, held in Delhi in 1950, the Indian National Science Academy set up a History of Science Board, which in 1964-65 became the National Commission for the Compilation of the History of Sciences in India. As a major step in stimulating academic and professional interest in this field, the commission had brought out in 1971, *A Concise History of Science in India* – a comprehensive volume encompassing the disciplines of astronomy, mathematics, medicine, chemistry and metallurgy, agriculture, botany and zoology – with contributions from specialists, and edited by D. M. Bose, S. N. Sen and B. V. Subbarayappa. This concise History surveyed the ground from pre-historic times up to the British period.

The present two-part monograph – a collaborative effort of Arun Kumar Biswas and Sulekha Biswas – has also been sponsored by the Indian National Science Academy, History of Sciences Division. The work that has been devoted to the subject of minerals and metals, their production and applications in ancient and medieval India, presents a detailed analysis of archaeological evidence and literary evidence (as available in Sanskrit), for the period up to 1400 AD.

The Biswas couple have brought their wide-ranging academic experience and scholarship to the formidable task they had set for themselves – of collection, collation, evaluation and construction of a vast body of historical material, to present substantial and valuable

conclusions. A. K. Biswas, who is presently Mahendralal Sircar Research Professor in History of Science, at the Asiatic Society, Calcutta, had been on the Faculty of IIT, Kanpur for over three decades (1963-95). His professional interests have been in the areas of applied chemistry, surface chemistry, mineral engineering and hydro-metallurgy. He has had a long-standing interest in archaeo-metallurgy and the history of science. His deep appreciation of India's cultural heritage has inspired him to author several books, including one on *Science in India*, and another on *Profiles in Indian Languages and Literatures*. Sulekha Biswas is a scholar in Sanskrit and has been a fellow-collaborator with Biswas, assisting him in the selection and interpretation of Sanskrit texts and treatises on science and religion.

Notwithstanding the overpowering volume and detail of material presented in the two volumes, what facilitates the reader and sustains the interest is the structured presentation. Volume 1 which presents the archaeological evidence is conveniently divided into 21 chapters. Both the preface to the volume and the introductory chapter outline the logic and methodology, and the research motivations. Following chapters two to seven, – dealing with the splendid variety of minerals, gem stones, metals, ornaments, tools and metal ware, and the associated technologies as unearthed at various excavation sites like Mehargarh (Baluchistan) (pre-Harappan), Mohenjo-Daro (Sindh, Pakistan), Harappa (Ravi basin), Chanhudaro (Sindh), Lothal (between Ahmedabad and Bhavanagar, India), and Ahar (near Udaipur, India) – chapters 8 and 9 are devoted to the Chalcolithic cultures of Peninsular India and Eastern India, and chapter 10 to a critical evaluation of copper technology in ancient India. Iron technology in ancient India is covered in chapter 12 and again in chapter 19 (the Delhi pillar, wootz steel and Damascening). There are individual chapters devoted to particular topics: Minerals and metals in the Mahabharata epic sites, in Taxila through the centuries, India's trade in minerals and metals, and mining in ancient India.

Arising out of his own analysis of all the source material, Biswas makes bold to express the firm views that there was

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a continuity of the civilization from the pre-Harappan to the post-Harappan era (there was no invasion from outside), and that both copper technology and iron technology were indigenous developments and not the result of any diffusion from other civilizations. He has asserted that he has been very objective in making these assessments and no chauvinistic sentiment has clouded his judgement.

One of the amazing achievements of ancient Indian metallurgy is the carbothermic reduction of roasted zinc blende (sphalerite) to distil zinc vapour and recover the condensed metal. A collaborative project among M/s Hindustan Zinc Ltd, the Department of Archaeology, Baroda University (K. T. M. Hegde) and the British Museum Research Laboratory (P. T. Craddock and colleagues) involving archaeological excavations in the Zawar region of Rajasthan, brought to light extensive debris of the ancient zinc smelting furnaces and retorts. Through techniques of ^{14}C dating, the activity has been placed in the first millennium BC. The primacy of India's contribution to zinc smelting is now well accepted. It was not till 1730 AD that a similar technology for zinc extraction was successfully attempted in the Western world (in England). Biswas, who had carried out investigations on the characterization of Zawar zinc retort residues, has dwelt at length on the scientific basis of the Indian process, in his chapter on 'Antiquity of zinc and brass in ancient India' (chapter 18).

Volume 2 is a search for and survey of references to minerals, metals, processes and related concepts in Vedic and Sanskrit literature up to fourteenth century AD. It is an eloquent illustration of the versatility of the Sanskrit language to develop an extensive vocabulary to distinguish various minerals and metals in relation to their appearance and properties, to develop scientific concepts, and to deal with the subtleties of materials technology. At the same time, the Biswas team has made the observation that the references to minerals and metals in the Sanskrit literature are surprisingly scanty and scattered, the exceptions being the cases of Panini's *Asthādhyāyī*, Kautilya's *Arthasāstra* and the *Rasasastra* texts.

As interesting examples of etymology, for cat's eye or beryl Panini uses

the description *Vaidūrya* (derived from the city of Vidura where the gem was cut). While *ayas* was used as a general name for metal, *Kālāyasa* or black metal referred to iron and *lohitāyasa* or red metal referred to copper. *Padārtha* (which literally meant the meaning of a word) was also adopted to mean a material or substance.

Arthasāstra is an important sourcebook on the economic importance attached to materials and metals in ancient India. While there has been some controversy on the date of the *Arthasāstra* – placing it between 4th century BC and third century AD – Biswas accepts the traditional view that it was the work of Kautilya (also known as Chānakya or Vishnugupta), in the time of Chandragupta Maurya whose reign commenced around 321 BC. Apart from giving details on metal processing, the *Arthasāstra* also defines the duties and responsibilities of the director of mines (*Ākarādhyaksha*) and the director of metals (*Lohādhyaksha*).

Volume 2 devotes a long chapter to gemmology literature (a millennium of *Ratnasāstra*) and a short chapter to non-gem minerals and metals in ancient Indian texts. Alchemy was pursued for a long time in India, perhaps even from the Ayurvedic period, but more prominently between fourth century AD till as late as the 14th century AD – but with greater emphasis on the use of minerals and herbs in health and medicine. Literature on Indian alchemy is voluminous, in the form of the *Rasasāstra* texts and is discussed in chapter 8 in this volume.

Rasaratnasamuccaya (compiled during the 14th century AD) has been described by the authors as 'a pinnacle in the Indian latro-chemistry'. It is interesting for the detailed descriptions related to mineral and metal processing. Extensive extracts referring to zinc extraction, brass and lead, and different kinds of iron are included in chapter 9, but the text is in Sanskrit, and the authors have not considered it necessary to provide English translations.

There is a closing chapter titled 'The future of the past' in volume 1, and a resume in Volume 2, where the authors have summarized the major conclusions from their work, and made suggestions for future work. Maps showing the sites of archaeological excavation, the chronological presentations of historical

information, the large number of tables giving the chemical analysis and other significant details of ancient objects, and the beautiful colour plates of jewelry, seals, implements, icons, metal work, stone sculpture, etc. all add great value to the presentation.

It is undisputed that Indian civilization – in the ancient and medieval periods – had touched great heights in the aesthetic application of minerals and metals, and in metallurgical accomplishments of a high order and pioneering quality. The authors have raised the familiar question why such a vibrant civilization did not maintain its leadership in the subsequent eras and did not proceed to develop modern science and large-scale technologies. (There are many aspects to this question as explained by B. M. Udgankar in his comprehensive article 'Scientific traditions and other traditions', *Curr. Sci.*, 1995, 69, 197–206.) The authors place particular emphasis on the stratification that had crept into society, where specific skills (like working with different metals: iron, zinc or gold) remained confined to particular castes and tribes, the intellectual class largely stayed aloof, and there was no effective cross-communication and discussion of experience.

The Biswas couple have produced a very impressive and valuable compendium of information, that should serve as a consolidated sourcebook for future historians and research workers.

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Parasitic Infections of Domestic Animals: A Diagnostic Manual, Johannes Kaufmann. Birkhauser Verlag, Basel, P.B.No. 133, CH-4010. SFr 68. 1996. 423 pp. Price: DM 78.

Several textbooks on parasitology and parasitic infections of domestic animals are available. These mainly focus on the parasites grouped according to zoological classification; give detailed descriptions of morphology and life-cycle; and small notes on pathology, symptoms and control. Some of the books contain

separate host-wise chapters on postmortem diagnosis and control, again under zoological grouping. Methods of diagnosis wherever mentioned are insufficiently described to be of much value to the field laboratory staff.

A few manuals extant on the subject contain accounts of diagnostic techniques and requirements for biological products of the listed diseases. Some manuals produced by commercial houses contain excellent coloured photographs of the parasites and lesions, but do not provide sufficient information on diagnosis and control.

All these are of limited practical help to field veterinarians and staff of field laboratories, in the diagnosis of the parasites involved. Kaufmann has addressed the problem of reaching rapid and precise diagnosis of parasites involved.

Kaufmann is eminently suited to undertake this stupendous task as he has the experience of dealing with the subject both in the laboratory (he heads the Diagnostic Section of the Institute of Parasitology at University of Berne, Switzerland) and in the field as he worked in a field laboratory in Gambia in late 1980s. Besides the material collected by him then and used in the book, he has incorporated in it a lot of information gleaned from about 300 institutions and universities. He is the principal author and has tapped the expertise of some 74 scientists based in Africa and Europe who collaborated as contributors or advisers, in producing the book. The result is a unique, beautiful, practical and self-contained manual for rapid diagnosis and control of parasitic infections of domestic animals and poultry.

Though the book was primarily meant for use in Africa, it is of equal relevance elsewhere for rapid diagnosis of economically important parasitic infections occurring worldwide. A few gaps in the information of local importance can be easily filled in. The book is unique as it deals with the parasites host-wise, system-wise and organ-wise as a veterinarian encounters them in the field. As he collects material from sick animals, or performs postmortem examinations, the book keeps pace with him by providing information as to which parasite(s) to expect, and in establishing rapid specific diagnosis.

The first chapter is on METHODS

for identification of parasites by direct and indirect techniques. The former includes up-to-date methods for examination of faeces, blood, skin scrapings and tissues. Detailed descriptions of these methods have been given, viz. counting of ova and oocysts and their identification, recovery of lungworm larvae, culture and recovery of third stage larvae of nematodes and their identification, haematology including quick staining methods, viz. Diff-Quick®, QBC®, dark ground/phase contrast buffy coat, and skin scrapings.

As most parasites produce antibodies in blood these are detected by indirect immunological techniques – indirect fluorescent antibody test (IFAT) enzyme-linked immunoabsorbent assay (ELISA), immunoblotting (Western blot) and complement fixation test (CFT) adequate descriptions of which have been provided. These are specific antigen-antibody reactions. Within the limitations, the tests are useful in diagnosis of parasitic infections.

The above-noted techniques meet routine diagnostic requirements of field workers. But for epidemiological studies, molecular biological techniques for detection and identification of parasites using nucleic acid probes come in handy. The last section of the chapter describes the techniques based on DNA probes and PCR (polymerase chain reaction) or both: DNA-random amplification of polymorphic (RAPD)-PCR. These techniques being extremely sensitive and highly specific are finding increasing use in diagnostic parasitology.

The next six chapters describe parasites of cattle, sheep, horses and donkeys, dromedaries, swine and poultry, respectively. Each chapter is divided into 5 sections representing stages in which parasites may occur: in gut and faeces, blood and circulatory system, urinogenital system, internal organs, and on body surface, respectively. Under each stage, the parasites are described under protozoa, helminths and arthropods. Information on the parasites is presented under sub-heads: location, hosts, species description (morphology and life-cycle), geographic distribution, symptoms, significance, diagnosis, therapy and prophylaxis.

Rickettsiaceae (*Ehrlichia* spp., *Anaplasma* spp., *Cowdria ruminantium*, *Eperythrozoon* spp., and others) – a group of parasite-like pathogens often

seen in blood or tissue smears are also described for differential diagnosis.

The book is parasitology made-easy for field workers. It is a unique and 'well-conceived manual, intelligently and copiously illustrated'. It has some 300 colour and 400 black and white illustrations of parasites, their developmental stages, lesions, affected animals showing cardinal symptom(s), schematic diagnosis of life-cycles, keys, etc. The text is precise and concise. All this facilitates rapid diagnosis of parasitic infections of domestic animals under field conditions.

The book has been 'very reasonably' priced due to generous financial aid from three Swiss institutions (Ciba, Swiss Development Corporation and University of Berne). This brings the book within reach of veterinarians, meat inspectors, and teachers and students of veterinary parasitology who need it most.

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The State of Food and Agriculture. Food and Agriculture Organization of the United Nations, Rome, Italy. 1995. 301 pages.

The 1995 State of Food and Agriculture Report of FAO has chosen agricultural trade as its special feature. The other sections are common to the different reports and provide an overview of the current agricultural situation in the world. The data provided by FAO constitute the most authentic information available to agricultural researchers and policy makers. The data are dealt with both from a global and a regional perspective. Of particular interest is the detailed analysis of the food security challenges facing India.

In the section dealing with India, emphasis has been placed on the 1990 economic crisis arising from the large foreign debt and repayment liability. The steps taken by Government since then have been summarized in a meaningful manner. However, the following developments after the introduction of

BOOK REVIEWS

the new Economic Policy in 1991 highlighted in the report merit careful consideration.

- The inward-looking, import substitution development strategy, which was aimed at rapid industrialization, shifted resources from tradable agriculture to industry by turning the terms of trade against agriculture.
- The overvaluation of the exchange rate subsidized imports and adversely affected all exports, especially agricultural exports.
- Most sector-specific policies at all stages of production, consumption and marketing of agricultural produce, worked against agriculture. For example, the price policy was in practice designed primarily to help the consumers. Farmers were generally given low administered prices in the name of helping the urban poor even when they had to pay higher prices for domestically produced inputs because of the protection given to local industry. In addition, a major proportion of the costs of the inefficient functioning of parastatal organizations, such as the Food Corporation of India, were borne by farmers.

The above problems continue to affect India's agricultural progress. The flow of credit to rural areas had gone down very steeply. It is only during the

last two years serious efforts have been initiated to reverse the drain of resources from the village to the town and city. Globalization of Indian agriculture offers both opportunities and challenges to policy makers. However, there is need for a coherent policy towards issues arising from globalization with particular reference to their impact on employment generation and rural livelihoods. Mass production technologies will kill the *production by masses approach*, which alone is relevant to our conditions of expanding population and shrinking per capita availability of land and water.

There is a detailed discussion on all aspects of agricultural trade. The report stresses that markets alone cannot ensure environmental quality and sustainable agricultural development. Private values often do not take account of social costs. The principles recommended for multilateral action under the World Trade Agreement include: non-discrimination, transparency, choice of measures that have minimum adverse environmental impact and commitment to the cause of eco-technologies. There is need to ensure a proper match between trade and environmental objectives.

As in the past years, the *State of Food and Agriculture* 1995, includes a computer diskette with time series data for about 150 countries.

In his foreword to the Report, Jacques Diouf, Director General of FAO has drawn attention to the World Food Summit scheduled to be held in November 1996 in Rome for the purpose of mobilizing concerted global action to

overcome problems of hunger and food insecurity. Diouf has pointed out that the deaths and sufferings associated with food insecurity are as unnecessary as they are intolerable. The end of hunger and malnutrition, far from being a utopian or poet's dream, is within the reach of our modern society's technology, resources and understanding of the underlying problems.

A similar conclusion was arrived at the Science Academies Summit on 'Uncommon Opportunities for a Food Secure World' held at Madras in July 1996. A summary of the conclusions of this Summit has already appeared in *Current Science* (1996, 71, 342-346). The Ten-Point Action Plan recommended by the Science Academies provides a blueprint for achieving food security at the level of each individual. There is no scientific, economic, environmental or political excuse for inaction in converting the opportunities opened up by modern science and technology for making endemic and poverty-induced hunger a problem of the past into field level accomplishment. The decision of the Government of Tamil Nadu to initiate a Hunger-Free Area Programme in the State is the first decisive step taken in our country to stress that ending hunger is an idea whose time has come.

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22-25 November 1996 – Orange County, Coorg District, Karnataka

This discussion meeting is intended to get together Indian scientists active in the area to discuss current research as well as possible future directions, and to encourage formulation of any joint projects that might be of interest to them. Speakers at the meeting will include Indian scientists pursuing research in stability and transition as well as some visitors from abroad, including Dr Jitesh Gajjar (University of Manchester), Prof. Michael Gaster (Queen Mary & Westfield College, London) and Prof. K. R. Sreenivasan (Yale University). A small number of places in the meeting will be available for students, university faculty and other scientists either working in the area or seriously intending to do so. If you are interested in attending the meeting, please send a copy of your bio-data with a brief statement of interest to

Prof. Roddam Narasimha
Centre for Atmospheric Sciences
Indian Institute of Science
Bangalore 560 012
(Fax: 080-334 1683, e-mail: roddam@cas.iisc.ernet.in).

Participants will be required to be present in Bangalore before the evening of 21 November 1996 and will be brought back to Bangalore on the evening of 25 November. Arrangements for transport to and accommodation at the venue of the meeting will be taken care of by the Indian Academy of Sciences.

Current Science

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1. Mukundan, T. and Kishore, K., *Curr. Sci.*, 1991, 60, 355-362.
2. Constantine, G., in *Biology of Bats* (ed. Wimsatt, W. A.), Academic Press, New York, 1970, vol. 1, pp. 319-322.

Acknowledgements should be brief. Footnotes are not allowed except to identify the corresponding author if not the first.

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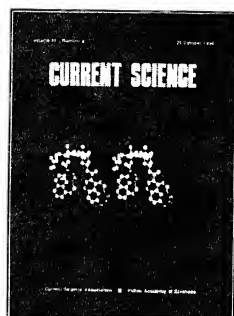
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The ever-ready pollen

Nature's oddities are often well beyond the best imagination of human mind. Some of these have indeed been the inspirations for a few of the most artistic and aesthetic creations by man. Often these oddities also pose clear challenges to his inquiring mind. Indeed as Stephen Jay Gould remarks, these oddities of Nature are the scientists' bread and butter. One such bizarre feature of the plant reproduction is reported in this issue by Sharma and Koul (page 598): the precocious germination of pollen grains in a species of *Trifolium*.

Normally pollen grain are released as well-protected units such that they could beat the odds of weather during their long journey, often hitch-hiking on the body of insects or birds or mammals from the anthers of a flower to the stigma of another flower of probably another plant. Obviously they are induced to germinate only by the uniquely suitable conditions prevailing on the target stigma. The conditions are so unique to each species that plant breeders have to frequently struggle to construct suitable growth media for germinating the pollen grains of different species.

However, occasionally in certain species the pollen grains are precociously germinated even before they are released from the anthers. Sharma and Koul report that in *Trifolium dubium* the pollen grains germinate well within the anther in all the anthers of all the flowers of all the spikes of all the plants they studied. They also found that in some situations the pollen tubes enter the stigma from the undehiscent anthers. They interpret it as a strategy to facilitate obligate self-pollination. This, however, is not consistent with their statement that the

'species practices high pollen competition'; one cannot visualize a severe level of pollen competition in a species that is obligate self-pollinated. There could be alternate explanation also: precocious germination of the pollen grains has also been interpreted as a strategy for gaining a head start in the severe competition among the pollen grains for the limited ovules in the ovary – a form of male-female competition in plants. This possibility is especially likely in *Trifolium dubium*, as there are only two ovules of which one at the base eventually aborts. Thus pollen grains would indeed be selected to be competitive to gain access to the ovule at the stigmatic end. But if this be true, Sharma and Koul might have to reevaluate the level of selfing in this species.

K. N. Ganeshaiah

Flow process modelling

Whether we like it or not, we have to face up to the fact that the whole world has now come under the spell of the American ethos. One of the more disturbing aspects of this ethos is the notion that one has to choose between selling and being sold, with no other alternative being available. In this harsh climate of hard sell, so many tall claims are made that we become very wary of any claim at all. Take the case of computational fluid dynamics (CFD). It has been very common among some of its practitioners (the school of *hard* CFD?), to claim that with sufficient computing power available CFD has made or will make expensive wind tunnels and experimentation obsolete; that given enough computing power, any fluid dynamic problem can be

solved. To more rational and knowledgeable people, these claims have sounded like sheer madness. But it would be very foolish to let the hysteria put one off from correctly estimating the true potential of CFD. What is its real potential?

The reason that fluid dynamics is so hard is because nonlinearity is at its core, and as we all know, there are no general ways of dealing with nonlinear problems. As a consequence, even after more than a century of effort, we still have no real handle on the central issue, of immense practical importance, of turbulence, which Feynman considered the last unsolved problem of classical physics. Since theory could do little, one was forced to rely on experimental testing to solve the kind of fluid dynamical problems that needed to be solved off campus. What is not often noticed or recognized, is that such experiments and testing were often ingenious and clever, often drawing, with the help of theory, useful conclusions of a general nature from a small number of tests; but there was no glamour. In this frustrating situation, enter that panacea of modern times, the computer. Suddenly, it appeared that people who knew little mathematics or physics or fluid dynamics could suddenly 'solve' difficult fluid dynamic problems and even present the results in the most attractive colours. It appeared that turbulence was licked or was about to be so. *By Golly, it could even be done on a PC!* Or so the claims went. But what was the truth? Yes, it is true that many problems can now be solved, especially for complex geometries and flows, that could not have been dreamed of a decade ago. More important, there are flows of great industrial importance that can now be modelled at least approximately, but usefully, by CFD using inputs

from experiments and theory. But the problem of turbulence has not been licked. The shameful truth is that we still cannot compute, from first principles, how much power we would need to pump a given volumetric flow of liquid through a pipe if the flow rate is high enough!

Yet great successes have been achieved and are being achieved. There is a large body of hard facts to show that in the aerospace industry, CFD is now playing a crucial role in design and development. I would go so far as to say that some of the planes flying today, would not have been the commercial successes that they have become if it were not for the economies that intelligent use of CFD brought about. Less well known is the role that CFD can play in non-aerospace industries. It is for this reason, especially in the Indian context, that Ranade's article (page 602) is timely and appropriate. At a time when Indian industry is being exhorted to be competitive in order to survive, when it is often suggested that Indian companies are backward in not supporting inhouse R&D, that no attempts are being made to improve existing technologies, let alone develop new ones. Ranade's examples and suggestions show us an alternate, positive path. By the intelligent use of modelling, of grafting on to existing codes

incremental new details and ideas, it is possible to make suggestions and predictions that could have a significant impact on technology and hence on the economy. Ranade points out, and I believe correctly, that important inputs will have to come from basic research if this programme is to succeed; further that this effort, in order to be really successful would have to be a collaborative effort of scientists working in the universities, the national labs and in industry. There is a lot more than hype here. Let us hope that the vision projected here comes to pass and that CFD comes to play the useful and key role that it is really cut out for.

P. N. Shankar

Designing molecular locks

Emil Fischer's 'lock and key' concept of molecular recognition is a century old. Spurred by the exquisite specificity of biological receptors (invariably macromolecules), chemists have in the last few years turned their attention to the design of molecular hosts which can recognize specific guests. The game is conceptually simple; design molecules which are most often hollow or concave and attempt to find guests which can then fit snugly into cavities stabilized by a variety of non-covalent interac-

tions. Specificity is then achieved by tuning cavity size and the nature of the binding interactions. While crown ethers, cryptands, cyclodextrins and cyclophane based systems were intensely investigated in the period between the mid 1960s and the late 1980s, attention has turned to diverse molecular scaffolds in recent times. A particularly versatile unit is the bile acid skeleton in which polar, functionalisable hydroxyl groups lie on one face of an amphiphilic molecule. Maitra (page 617) provides an elegant account of the use of bile acids in generating novel, artificial receptors and as auxiliaries in asymmetric organic synthesis. Laboratory design is still far from achieving the specificity and versatility of biological receptors. 'Molecular locks' are frequently the products of sophisticated design strategies. The interactions which bind the lock and key together are hard to control, except in the case of hydrogen bonds. The organic chemist is, however, not really bound by the limitations of biochemistry. The expectation is that imaginative design approaches will lead to novel molecules which will have diverse applications including use as molecular devices and sensors.

P. Balaram

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CORRESPONDENCE

Biotechnology

The views expressed on Biotechnology by A. S. Rao (*Curr. Sci.*, 1996, **70**, 955–956), P. Tauro and M. V. Nayudu (*Curr. Sci.*, 1996, **71**, 169–170) are timely, thought provoking and highly relevant to the progress of Biotechnology in our country in future years.

Biotechnology being a multidisciplinary subject, requires coordinated efforts and expertise of scientists with different backgrounds to be put together in order to achieve success. I had stated in my earlier article (*Curr. Sci.*, 1994, **66**, 137–140) that Biotechnology is an 'Olympics' involving the interplay of Biology, Chemistry, Physics, Mathematics and Engineering – particularly in developing technology out of microbial fermentations and manufacturing products on commercial scale. As such no one can be a complete 'Biotechnologist' with absolute mastery in all the disciplines, but one can form a part of the networking that is essential for achieving realistic goals in commercializing Biotechnology.

Biology is essentially the basic science on which biotechnology progress depends and in the case of Microbial Technology, Microbiology is the bedrock from which newer developments in Biotechnology would emerge. I believe and I have emphasized on several occasions that natural microbial biodiversity is the resource pool for discovering novel bioactive metabolites and there is an urgent need for exploring natural resources for novel microorganisms by innovative techniques and conserving them in well-organized germplasm banks. In combination with application of modern techniques of genetic engi-

neering and recombinant DNA technology including PCR, gene amplification, etc., unlimited potential for developing new technologies based on indigenous discoveries would emerge and make our research and technology competent and globally competitive.

The question to analyse and answer is how far are we presently prepared to accept this challenge in our country and are we doing the right things to meet the challenges in the 21st century – with the GATT, WTO and IPR coming into full swing when we have to play our role and achieve international competitiveness in Biotechnology.

In my opinion, the following are the most essential points on which focussed decisions need to be taken and implemented fast to achieve this competence and competitiveness.

We need to establish active schools of research at Universities and national institutes to explore, identify and conserve in pure culture germplasm banks of indigenous microbial strains which must represent biodiversity of the native microflora from the diverse ecosystems which our country is abundantly endowed with. Among microbes, expertise in the taxonomy, physiology and biochemistry of fungi and actinomycetes is either non-existent or will be vanishing as the older generation of microbiologists fade off and these areas need the proverbial 'shot in the arm' urgently. Research work and capability build up in the modern aspects of genetic engineering must keep pace with the positive developments in the developed world – for example the present use of filamentous fungal systems with their

capacity for protein hypersecretion as the preferred hosts for heterologous expression of genes (the 'Cassette Expression System') which we need to develop expertise in and apply to our future technologies in bioproduct manufacture.

We need to establish the infrastructure as well as the technical competence of germplasm banks conforming to international standards on a priority basis. Culture collection(s) so established must get recognition under the Budapest treaty to enable our biotechnologists in future years apply for international patents without the necessity of having to deposit our patent strains in other recognized culture collections abroad. National patent laws including the patentability of both naturally occurring and recombinant strains must be debated in depth and policy decisions quickly taken and speedily implemented.

In the light of some of the points discussed, human resource development for biotechnology becomes most vital for future progress. The culture of working in teams respecting one another's competence and expertise and putting the heads together to work out new problems and find innovative solutions through individual and collective creativity is most essential for Biotechnology as a science to blossom into world class technology in our country.

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In situ pollen germination and selective ovular abortion in *Trifolium dubium* Sibth

Trifolium dubium Sibth, an exotic leguminous weed, practices a special pollination mechanism and non-random pattern of ovule abortion.

A procumbent annual herb, the plant bears lemon yellow flowers in compact hemispherical spikes. Each plant is profusely laden with flowers. Individual plants bear 41–54 corymbose heads. The plants range between 28 and 35% in their reproductive effort (as per dry biomass estimations).

Reproductive apparatus of the species comprises 10 diadelphous stamens (9+1), and a small, 640 µm long pistil. The stigma is papillate and wet. When receptive, it gets covered with copious,

lipoidal secretion which stains positive with Sudan-black B. Although small, the style is so curved as to bring the stigma close to anthers. The ovary is unilocular with two ovules.

The species is obligately self-pollinated. Every anther within a flower, every flower of a spike, every spike on a plant and every plant in a population practices *in vivo* pollen germination. Pollen grains (40–60 per anther, $\bar{X} = 49.14 \pm 1.08$) germinate inside anthers when the latter are close to stigma. Anthers dehisce following secretion of stigmatic exudates. The stigma surface gets covered with hundreds of germinating pollen grains

(Figure 1). Occasionally, anthers get detached from filaments, clog the stigma, and cover the stigmatic surface with germinating pollen grains and pollen tubes emanating therefrom. In some plants only pollen tubes come out of the anthers of intact stamens and enter the stigma. Pollen tubes traverse the style in bundles. Later, they grow along the ovary wall and fertilize both the campylotropous ovules, within the unilocular ovary. The species practices high pollen competition with about 250 pollen grains available for siring one ovule.

Following fertilization, both ovules register increase in size. The rate and extent of increase is always significantly greater in the ovule closer to stigma. The basal ovule always aborts. The site of collection of plants and their age notwithstanding, the pattern of ovule abortion is always the same. All mature pods are therefore, single seeded. Seed abortion is obviously non-random.

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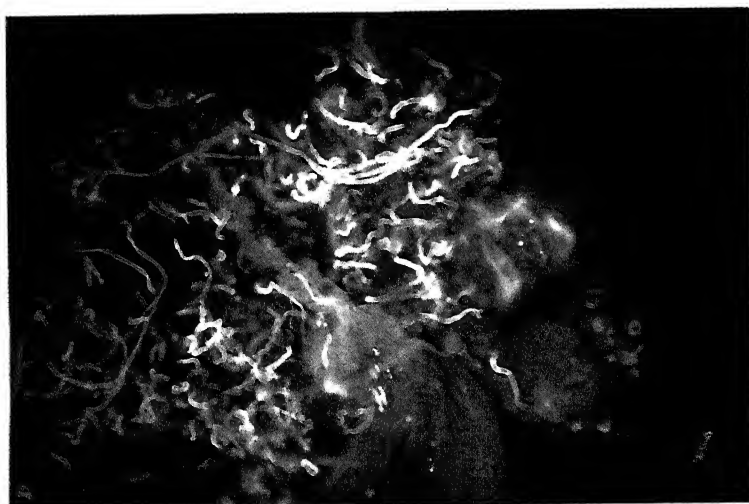


Figure 1. Stigma surface clogged with germinating pollen grains emanating from anthers.

Discotic liquid crystals – Self-organizing molecular wires*

Neville Boden, Richard Bissell, Jonathan Clements and Bijan Movaghar

WHEN considering the possibility of commercially viable applications for discotic liquid crystals, it is worth noting that in the case of calamitic liquid crystals some 80 to 90 years elapsed between their discovery in 1888 and their first application in displays. Since discotic mesophases were only discovered in 1977 (ref. 1), it is not surprising that the first applications are yet to be realized.

Recent research has established that the individual molecular columns in discotic liquid crystals can act as molecular wires for the transport of charge^{2,3} or excitons^{4,5}. This unique property is set to give rise to commercial applications in fast high-resolution light scanning and xerography, and in environmental gas sensors in the immediate future, and, in the longer term, to hybrid computer chips which will enable us to communicate electronically with molecules.

The reasoning behind this optimistic vision of the prospects for discotics is discussed in the context of recent and current research.

Discotics as arrays of molecular wires

The high mobility of charge carriers along the individual molecular columns (wires) in discotic liquid crystals stems from their unique architecture. They are comprised of disordered stacks of disc-shaped molecules, such as the hexaalkoxytriphenylenes, arranged on a two-dimensional lattice (Figure 1). The separation between the aromatic cores is of the order of 3.5 Å, so that considerable overlap of π^* orbitals of adjacent aromatic rings occurs. This produces a quantum band structure along the columns with a band gap of 2–4 eV and a band width of approximately 0.1 eV. Charge carrier mobilities are in the range 10^{-2} to 10^{-5} cm² V⁻¹ s⁻¹ and typically at least 10^3 greater than the corresponding mobilities in the perpendicular direction. The perpendicular transport is fluctuation controlled or impurity assisted.

Long-range one-dimensional transport is generally not realistic in molecular solids due to the presence of structural defects, or deep traps. However, in discotic mesophases the liquid-like fluctuations in columnar or-

der, which take place on a time scale of less than 10^{-5} s, provide a 'self-repairing' mechanism.

This combination of properties is quite unique and opens up new opportunities for applications of liquid crystals.

Applications

Fast, high-resolution xerography

Recently it has been demonstrated that mobilities for photoinduced charge carriers in the columnar phases of pure, triphenylene-based discotic liquid crystals are of the order of 10^{-4} to 10^{-3} cm² V⁻¹ s⁻¹ (refs 3, 6) and are as high as 0.1 cm² V⁻¹ s⁻¹ in the highly-ordered helical phase of hexahexylthiotriphenylene⁷. This makes them suitable for use as the active charge transport layer in fast and high resolution xerographic and laser printing applications.

For the last 30 years, polymer organic photoconductors have been widely used as photoconductive media in the field of xerography and laser printing. The efficiency of photocopiers and laser printers is primarily due to the mobility of charge carriers in the photoconductive layer. Polyvinylcarbazole (PVK), for instance, exhibits a mobility in the range of 10^{-6} cm² V⁻¹ s⁻¹. However, this is too slow for application in fast laser printing and photocopying, and toxic inorganic compounds of selenium and tellurium are therefore used.

The surface field effect gas-sensor

The present generation of 'electronic noses' are based on an array of 16–32 individual gas sensors, each made from a conducting organic polymer which displays reversible changes in conductivity when exposed to polar volatile odours. Although the response is non-specific to individual odour molecules, a characteristic odour 'fingerprint' is obtained from the response of an array of sensors. However, a serious limitation of conducting polymers is the lack of response to non-polar molecules.

The surface of a discotic liquid crystal has a unique topology (Figure 2). Fluctuations in the lengths of the columns result in a 'conductive' surface layer. The

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*Reproduced from *Liquid Crystals*, 1996, 6, 1–4.

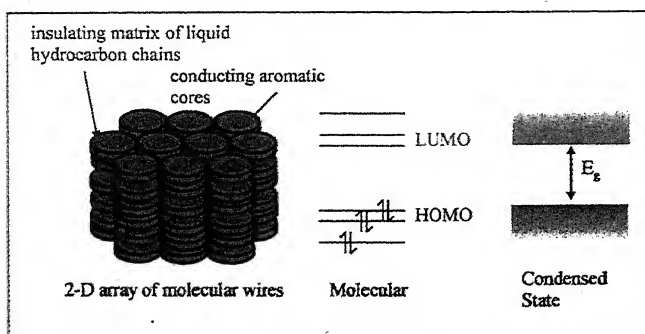


Figure 1. A schematic view of both the D_h phase in discotic liquid crystals and a simplified electronic structure of an isolated molecule and the condensed state showing the band gap E_g .

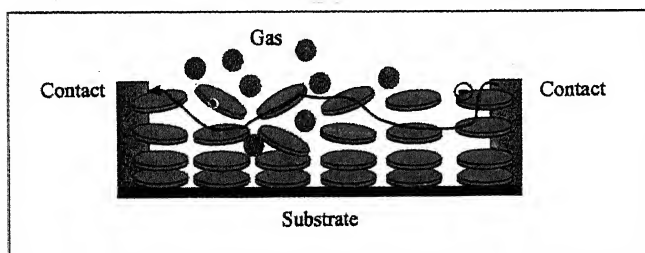


Figure 2. A schematic illustration of a gas sensor exploiting the surface conductivity of the discotic liquid crystalline film.

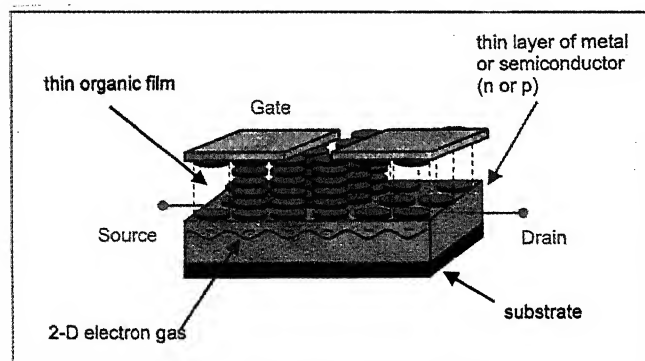
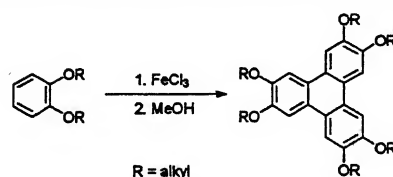
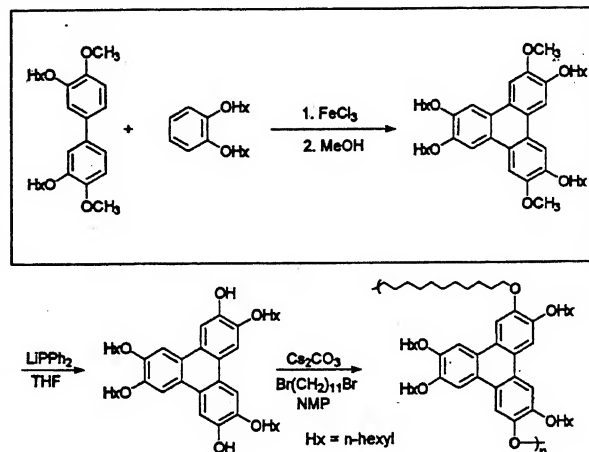


Figure 3. A schematic view of a hybrid discotic liquid crystal/silicon field effect transistor. The molecular wires generate a 2-D band structure in the silicon inversion layer. The source-drain current is therefore sensitive to signals along the columns produced by sensing molecules absorbed in the film.

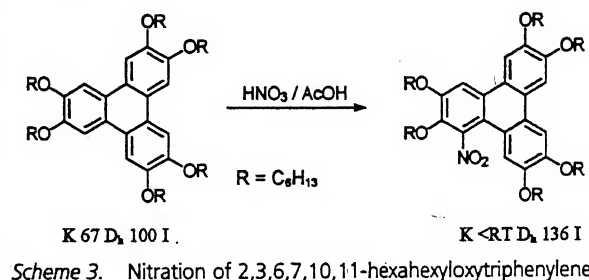
carrier mobility along the surface is fluctuation assisted and the tunnelling rates are exponentially dependent on the molecular core spacings. The core-core separation fluctuates with the surface and changes as soon as the surface is disturbed: i.e. locally melts, freezes, reorients, etc. The electrical conductivity of this surface layer is, therefore, very sensitive to the absorption of molecules. Discotic liquid crystals can therefore be used



Scheme 1. Synthesis of 2,3,6,7,10,11-hexaalkoxytriphenylenes.



Scheme 2. Synthesis of unsymmetrical triphenylene polymer precursor.



Scheme 3. Nitration of 2,3,6,7,10,11-hexahexyloxytriphenylene

as very sensitive gas sensors for both polar and non-polar molecules. The response has a physical origin and does not rely necessarily on charge transfer between the adsorbed molecule and the discotic aromatic core.

Communicating with molecules

The narrow electronic band structure, and the slow speed of response in discotic liquid crystals rule out many traditional semiconductor applications. We can, however, envisage applications for hybrid systems which combine the unique electronic properties of discotic liquid crystals with current silicon technology (Figure 3). Discotic liquid crystals form high-quality insulating films and could, therefore, replace the traditional oxide insulating gate in a field effect transistor. The novel feature now is that self-assembled molecular

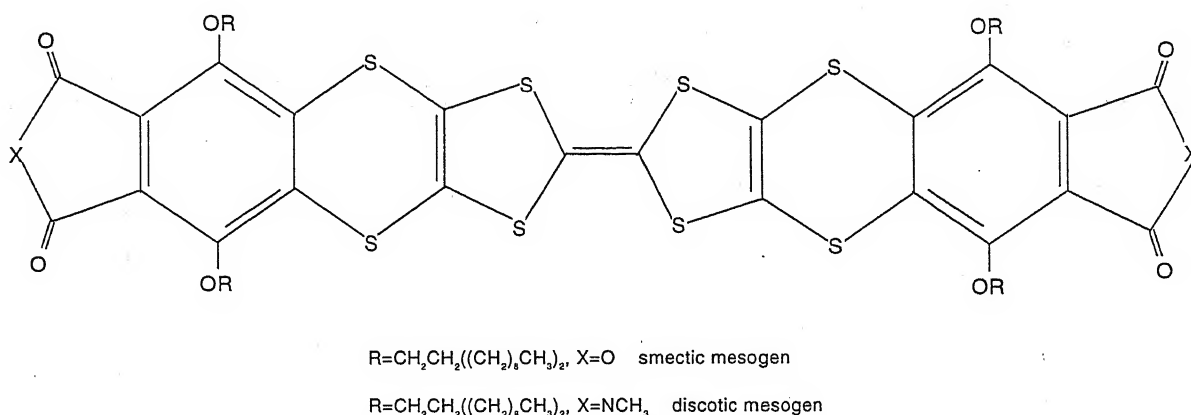


Figure 4. Examples of smectic and discotic tetrafulvalene mesogens.

wires in the liquid crystal can address the 2-D electron gas in the inversion layer of the silicon. This coupling is strong enough to give rise to a hybrid band structure in the inversion layer with the effect of amplifying perturbations in the molecular wires via restrictions imposed on quantum pathways. In this way we can transform molecular signals arriving in the molecular wires into electronic signals in silicon chips. Once in the chip, molecular signals, in the form of local current changes, or channel switching events ('on-off'), can be processed in the usual way.

Developments in design and synthesis

In our work we have used mainly hexaalkoxytriphenylenes. They are stable to heat, light and redox processes and their chemistry is fairly accessible. We are now able to produce large quantities of these materials of high purity and at an economic price. Routes for synthesizing low molar mass discotics⁸ (Scheme 1), polymeric discotics⁹ (Scheme 2), and for introducing substituents into the triphenylene ring¹⁰ (Scheme 3), are now available. The latter enables the electrical properties and phase behaviour to be fine-tuned. Device applications of discotic liquid crystals require the development of materials which are liquid crystalline at room temperature and can be easily processed as polymers.

Smaller band gaps can be achieved by using porphyrin, phthalocyanine or larger delocalized aromatic cores. Tetrathiafulvalene (TTF) and its many derivatives are principally studied in the context of solid state organic metals and superconductors. Significantly, we have recently made derivatives of (TTF) which, depending on

minor modifications to the core structure shown in Figure 4, form either smectic A or columnar discotic phases. In the former phase type there is no evidence of π - π stacking between the TTF cores and hence no pronounced electronic band structure in the material. The discotic phase, however, possesses ordered columns of TTF molecules arranged on rectangular lattice. We are currently investigating the electrical properties of this remarkable material. Thus by intelligent design, it is possible to introduce exciting functionality into discotic phases with the prospect of new applications for liquid crystals.

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Modelling of industrial flow processes: Opportunities for performance enhancement

V. V. Ranade

Detailed flow modelling of industrial processes offers new possibilities for performance enhancement and innovation in design of process equipment. In this review, we describe the overall methodology of industrial flow modelling. Some examples of applications of flow modelling are described to illustrate the methodology. Challenges and opportunities with reference to the Indian context are discussed.

ALMOST all the processes relevant to the manufacturing industry (chemical, petrochemical, fertilizer, metallurgical, power, cement, etc.) involve flow of fluids in some way or other. The innovation and competitive edge of any manufacturing industry, therefore, rests on how well these flow processes are designed and operated. If the underlying flow processes are adequately studied and controlled, there is always scope for performance enhancement and for evolving innovative design solutions. There are many instances in the past where innovative analysis and clever engineering of flow processes have realized substantial enhancements even in the so-called 'mature' technologies. One of the most striking examples of using knowledge of fluid dynamics to substantially enhance the performance of the reactor is the development of super-condensed mode of operation of fluidized bed polymerization reactors¹. With the right selection of nozzle design and nozzle locations, it is now possible to increase the capacity of such polymerization reactors by 50 to 100% (this means producing fifty thousand to one lakh tonnes per year of polyethylene more from the existing reactor!)

Performance enhancement of existing or new processes may be realized in a variety of ways such as producing more from existing equipment, producing better quality products, having lower energy consumption and more safety of operations with less pollution and so on. Realization of enhancement in any of the aspects mentioned above requires expertise from various fields ranging from chemistry and catalysis to reaction engineering, fluid dynamics, mixing and heat and mass transfer. For the given fixed chemistry/catalysts, the performance of industrial processes or equipment is a complex function of the underlying transport processes. These transport processes are in turn governed by the fluid dynamics and therefore on a variety of design and

operating parameters of process equipment. Specialized techniques and tools (of flow modelling) are therefore required to understand these complex flow processes.

Traditionally, experimental and semi-theoretical methods (like cold flow simulations or tracer studies) have been used to obtain the information about the fluid dynamics and mixing required for the process optimization. The information obtained from these methods is usually described in an overall/global parametric form. This practice conceals detailed local information about turbulence and mixing which may ultimately determine the process/equipment performance. With the emergence of high performance computers and advances in numerical techniques and algorithms, a relatively new approach to flow modelling of process equipment, based on computational fluid dynamics (CFD), is now available to engineers. This approach was mainly used by aerospace engineers so far. However, there is enormous potential of using it for many other manufacturing sectors including chemical, automobile, steel and cement. CFD deals with the solution of fluid dynamic equations on digital computers.

Computational modelling of industrial flow processes allows detailed analysis, at an earlier stage in the design cycle, for less money, with lower risk and in less time than experimental testing. Simulations have added advantage in that, diagnostic 'probing' of a computer simulation does not disturb the flow and usual operation! Various alternative configurations can be screened quickly using the validated CFD model. The detailed predicted flow field gives an insight into fluid behaviour and can sometimes give information which cannot be obtained from experiments. Therefore computational fluid dynamics (CFD) models are proving to be powerful aids in design and analysis of industrial flow processes. A critical review of CFD techniques and their potential and use for realizing performance enhancement will be useful at this stage. In this article, we discuss various aspects of modelling of industrial flow processes and its potential for enhancing performance of existing

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and new process equipment. Some examples of applications of flow modelling from our own research and consulting experience are described to illustrate the methodology. Challenges and opportunities with reference to the Indian context are discussed.

Computational fluid dynamics (CFD) framework

Computational fluid dynamics framework here means a complete tool for predicting the flow characteristics of the desired equipment. Various aspects of CFD framework along with key references are shown in Box 1. Several reviews describing the details of these aspects have been published (for example, refs. 2, 3). Here, we discuss some of the major issues without describing the mathematical details of specific formulations.

Transport equations

The closeness of predictions of the CFD model with the real flow behaviour depends on how well the user has represented the underlying physics in the mathematical formulations. Most of the industrial flow processes relevant to the process engineers, are complex and cannot be represented rigorously. The user, therefore, must appreciate the implications of the assumptions on which the formulation of the transport equations is based. Here we restrict ourselves to discussing major issues in the formulation of transport equations relevant for turbulent, dispersed multiphase flows, which are often encountered in chemical processing equipment.

Turbulence is often employed in industrial equipment to enhance the rates of transport processes. Turbulence is a three-dimensional, time-dependent, nonlinear phenomenon. The instantaneous velocity field in a turbulent flow is described by the Navier–Stokes equations. However because of the existence of extremely wide range of space and time scales in turbulent flow, direct numerical

simulation of turbulent flows is possible only at relatively low Reynolds number and that too if the geometry is simple. For most engineering applications it is still necessary to use turbulence models along with time averaged Navier–Stokes equations. It must be realized that most of the available turbulence models obscure the actual physical processes like eddies, high vorticity regions, large structures which stretch and engulf and so on. However, the cautious application and interpretation of turbulence models have proved to be a valuable tool in engineering research and design, despite their physical deficiencies.

A turbulence model is a set of equations which express relations between the unknown terms appearing in the time averaged Navier–Stokes equations and the known quantities. Two equation turbulence models are the simplest ones that promise success for flows in which length scales cannot be prescribed empirically. The k - ϵ model is the most widely tested model for a variety of complex flows. Many modifications such as multiple scale k - ϵ models or extra terms to compensate the shortcomings of standard k - ϵ model have been developed. At present, the two-equation model forms the basis for most engineering simulations of complex flows. More advanced models which do not use the assumption of isotropic turbulent viscosity or the concept of turbulent viscosity itself have been developed (algebraic stress models and Reynolds stress models). Recently, renormalization group theory based (RNG) models of turbulence have been developed^{4,5}. In RNG based k - ϵ model, the values of model parameters are evaluated by the theory. Moreover, the modifications of standard k - ϵ model like low Reynolds number modification, extra term of rate of strain are given by the RNG theory. While these models have not yet been sufficiently tested for engineering flow simulations, the initial results are promising. Considering that the framework of the standard k - ϵ model can be readily extended to RNG based k - ϵ models, the standard k - ϵ model is recommended for initiating simulations of industrial flow processes.

Multiphase flows are encountered in a variety of important industrial processes. For example, dispersed two phase flows are involved in steel making, cement manufacturing, polyethylene and polyvinyl chloride manufacturing, the fertilizer industry and in thermal power stations. It is indeed necessary to adequately represent the underlying physics of interaction of multiple phases in their mathematical models. An extensive literature exists on the modelling of dispersed two phase systems (reviewed recently by Ranade³ and Sommerfeld⁶ among others). There are two principally different approaches to simulate dispersed two phase flows, namely, the Eulerian/Lagrangian and the Eulerian/Eulerian approach. When dispersed phase hold-up is not very small and dispersed phase is introduced through a distributed inlet

Box 1. Computational Fluid Dynamics Framework

• Transport equations

- + Modelling of turbulence (Refs. 5, 30, 31)
- + Modelling of multiphase flows (Refs. 3, 6, 9, 32)
- + Modelling of rheologically complex fluids (Ref. 11)

• Numerical solution of transport equations

- + Discretization schemes (Refs. 12–14)
- + Solution algorithm (Refs. 14, 15, 33)
- + Solution of algebraic equations (Ref. 34)

• Computer codes for CFD simulations

- + In-house codes (Refs. 16, 17)
- + Commercial codes (Ref. 18)

rather than a single nozzle, it is easier and more efficient to use Eulerian/Eulerian approach in which both the phases are treated as continua.

Interphase coupling terms make two phase flows fundamentally different from single phase flows. In addition to the standard drag force, there are several other forces like virtual mass force (arising from the inertia effect) and Basset force (due to the development of a boundary layer around a bubble) which appear in the interphase coupling terms. An order of magnitude analysis presented by Hunt *et al.*⁷ suggests that for large vessels ($D > 0.15$ m), where the square of the terminal rise velocity of bubble would be smaller than the product of the gravitational constant and characteristics length scale, the interphase coupling term will be dominated by the drag force term. Various correlations are available to estimate the value of drag coefficient appearing in the interphase drag force term⁸.

For turbulent two phase flows, one can either use a standard Reynolds averaging procedure or one can use a practice similar to 'Favre' averaging in compressible flows. The time averaging of interphase interaction terms is tedious and involves several correlations. Johansen⁹ has derived an approximation for the low dispersed phase holdup case with the particles following Stokes law considering all contributions (drag, virtual mass, history term and lift force). The assumption of gradient transport can be used to model the correlation between fluctuating velocity and dispersed phase holdup. The gradient transport is strictly valid only when the size of the energy containing eddies is much smaller than the distance over which dispersed phase hold-up varies significantly. It should be noted that in principle, the value of turbulent Schmidt number used in such gradient transport will depend on the size of dispersed phase particles and integral scale of turbulence. Turbulent eddies smaller than dispersed phase will not contribute to the dispersion of dispersed phase particles. At present, however, there is no systematic data or theory available to estimate the values of Schmidt number for turbulent, two phase flows. The user, therefore, has to exercise his/her discretion in setting these values and interpreting the results.

There are a variety of different flow processes, not covered in the above discussion. For simulation of granular flows, additional modelling of stress in moving assembly of solid particles, viscosity of solid phase and effective pressure for solid phase need to be incorporated in the flow model. There is also a large class of industrial flow processes which involves laminar flows of rheologically complex fluids like polymer melt, inks, paints and detergents. Several studies and reviews on modelling of different classes of flows (for example, Gidaspaw¹⁰ for granular flows and Crochet *et al.*¹¹ for non-Newtonian flows) are available. Without describing

these issues in detail, we now discuss some aspects of numerical solution of transport equations.

Numerical solutions of transport equations

Numerical solution of transport equations mainly comprises the discretization of the governing equations and the solution of the resulting algebraic equations. Discretization of the governing partial differential equations can be done using finite difference, finite volume or finite element frameworks. The finite element methods have not yet been rigorously tested for multiphase flows in three-dimensional domains. The memory requirements of these methods are also much larger than the corresponding finite volume methods. For equipment engineering applications, conservative form of the governing equations discretized using finite volume methods is most appropriate (at least at present) from the point of view of accuracy and economy of computational resources.

The accuracy of a numerical solution depends on how close by the discretized equations represent the original partial differential equations. The desire to use higher order approximations for discretization must be balanced against the limitations imposed by the complexity of the problem being solved, the availability of the computing resources, stability and convergence of the solution algorithm and capacity to tolerate unphysical over and undershoots. Among the various schemes, QUICK (or in modified form of SHARP¹²) and second order upwind differencing schemes¹³ look more attractive from the point of view of accuracy and ease of implementation. In complex multiphase flows, a hybrid or power law scheme¹⁴ is recommended at least at the beginning.

The selection of algorithm for solving the transport equations requires consideration of the coupling and signal propagation among the equations (and boundary conditions). It must be realized that there is no single best algorithm for all types of problems. One has to make a choice by evaluating convergence performance and ease of implementation. For multiphase flows, additional coupling between two phases through interphase forces need to be handled. Spalding¹⁵ has proposed an inter-phase slip algorithm (IPSA) for this. In most of the two phase flow cases, the choice of algorithm for pressure-velocity coupling is often dictated by transparency and ease of implementation. After selecting a suitable algorithm for treating velocity-pressure coupling, the task that remains is to devise a suitable method to solve finite difference equations to obtain values of variables at all grid nodes. Because of interconnectedness and non-linearity, the task must be performed by iterative methods. Various methods such as point by point iteration (Gauss-Siedel or successive over-relaxation), linear

Gauss–Siedel and strongly implicit procedure (SIP) have been used for solving single phase flows. Convergence of pressure correction equation is often a rate limiting step especially for single phase flows. The rate of convergence depends quite strongly on the choice of under-relaxation parameters. Various techniques including methods based on additive correction philosophy have been proposed to enhance the convergence rates. Recently multigrid techniques have been shown to be very efficient in enhancing the convergence rates. Existence of dispersed phase raises further hurdles in the convergence of the iteration procedure because of additional coupling through the interphase terms. Spalding¹⁵ has proposed partial elimination algorithm (PEA) to treat the interphase coupling efficiently. PEA involves manipulation of finite difference equations of the velocities of both the phases at the grid node to eliminate the velocities of the second phase from the finite difference equations.

Computer codes for CFD simulations

It is necessary to translate the already described solution procedure into computer codes to generate useful simulations of engineering equipment. A CFD code needs to be designed to give appropriate importance to general applicability, ease of use and economy of computations. Recently Cross *et al.*¹⁶ have discussed the trends in CFD software engineering which are useful for the few code developer.

Our group (iFMg) has developed a CFD code called *SPARE* (for *SP*Arge*d RE*actors) based on a partial elimination algorithm (PEA) and an inter-phase slip algorithm (IPSA, ref. 15) along with the SIMPLER algorithm¹⁴. The sets of discretized equations for each variable are solved iteratively by means of an ADI technique. The non-linearities in the phase momentum and turbulence equations are handled by standard under-relaxation techniques. The core subroutines and problem specification routines have been organized in separate groups. The major routines are designed in such a way as to enable the user to construct various algorithms by modifying just one program. All the empirical information and boundary conditions can be specified through a single routine¹⁷. The performance of *SPARE* has been extensively tested both qualitatively as well as quantitatively³.

Instead of expanding the capabilities of an in-house research purpose code to carry out complex engineering flow simulations, it might be more efficient to use a commercially available general purpose CFD code. The development of a suitable mathematical model can be done using an in-house CFD code. The validated model can then be incorporated within the commercially-available code to carry out real life simulations. A num-

ber of CFD codes are available commercially (recently reviewed and compared by Dombrowski *et al.*¹⁸), each with its own particular set of features. Most of these codes provide user-friendly facilities for modelling complex geometries and grid generation. The powerful postprocessing facilities developed by professional programmers also aid user interpretation of simulated results. It should be noted here that though a variety of ready-to-use commercial codes are available, the experience and insight gained through the use of in-house codes turns out to be very valuable. The most important feature of the CFD code from the point of view of industrial application is the ability to incorporate or extend the code via user-written modules. Because no matter how general the code is, it will be necessary to develop specific submodels to simulate specific processes. Many of these commercial codes provide the ability to incorporate user-defined physical property models, source terms and new numerical features.

Methodology of flow modelling

Steps in a typical flow modelling project are described in Box 2. The first step of any flow modelling project is to relate the fluid dynamics with the overall objectives of the project and its implications for the performance enhancement. The complexity of the flow modelling tool can then be decided to achieve the set objectives. It is also essential to understand and quantify various time and space scales and the geometric complexity of the

Box 2. Typical flow modelling project

- **Problem definition**
 - + Identify key processes governing the overall performance
 - + Relate these processes with fluid dynamics of the process equipment
- = **Interaction with design and operating teams**
- **Development of generic flow model**
 - + Turbulence
 - + Multi-phase flows
- **Development of specific sub-models**
 - + Reaction source terms
 - + Physical property models
 - + Interphase transport models
- **Mapping these models onto CFD solver codes**
 - + Grid generation
 - + Solution parameters
 - + Post-processing strategies
- **Validation of the CFD model**
- **Application for design and process optimization**

system. This will enable one to represent the problem at hand in a mathematical framework. After finalizing the objectives and mathematical model, the next step is to map this mathematical model onto the computer code. The mapping of flow model mainly consists of specifying the geometry of the equipment under study, generation of appropriate grids within the solution domain, setting terms in the transport equations included in the CFD code or supplying additional problem-specific submodels to the CFD code and specification of corresponding boundary conditions. Prior knowledge of the various scales and likely regions of steep gradients will help in generating a suitable grid for the problem at hand. While generating the grids, extremes of aspect ratios and skewness should be avoided. The grid orthogonality has relatively little impact on flow accuracy in general, provided the angle between grid lines is not too small.

Once the appropriate grid is generated, the user has to select/specify the necessary information about the properties of fluids like molecular viscosity, density, conductivity. The correct specification of boundary conditions on the edges/external surfaces of the solution domain is crucial for the correct mapping of the problem. Care must be taken to understand and eliminate the influence of the location of outflow and inflow boundaries on the predicted flow results. Wall functions are routinely used to provide boundary conditions near the wall for turbulent flows which avoid the necessity of using very fine grids near the walls. However, one must be careful about such usage especially when heat or mass transport from the wall is important or when the accuracy of calculation of forces exerted by fluid on walls is important.

Once the problem has been mapped onto the CFD code, a user is interested in knowing details of simulated results. However, it is extremely important to understand the sensitivity of the predicted results to a variety of parameters like grid spacing, time step, boundary conditions and so on. This will normally involve some preliminary simulations to arrive at desired values of a variety of parameters involved in the simulations. It is often convenient to use grid sequencing techniques to solve complex problems. Normally, converged results can be obtained rapidly for the small number grids even with zero initial guess for all over the solution domain. These results can then be interpolated and used as initial guesses for the finer grids. It is also often desirable to increase the complexity of the problem in steps after starting with the most simplified situation. Underrelaxation factors for the different variables need to be employed appropriately to enhance the overall convergence rates. Patankar¹⁴ has listed many suggestions in this regard. The monitoring of convergence for each equation can also give clues about how to enhance the overall convergence rate of a CFD code.

Once the results are obtained on a sufficiently fine grid, it is necessary to formulate effective post-processing strategies to display these numerical solutions so that many aspects of the flow structure can be carefully studied. Appropriate validation exercise can then be carried out to verify whether the CFD model has captured the important flow characteristics of the problem at hand. The validated flow model can then be used to understand the various features of fluid dynamics and its relation to the process performance. The insight gained through such an exercise may lead to the evolution of better configurations or better operating protocols which will eventually lead to enhancements in process performance.

Flow modelling examples

Mixer design

Stirred mixers are among the most common process equipment in the chemical and allied industries. In such mixers, the contained fluids are circulated by the rotating impeller. The flow around the impeller interacts with stationary baffles and generates complex, three-dimensional, inherently unsteady flow. The fluid dynamics and therefore, the performance of the mixer depend on a variety of design and operating parameters such as size, number and shape of impeller blades, location of impeller(s), number of impellers, etc. Considering the

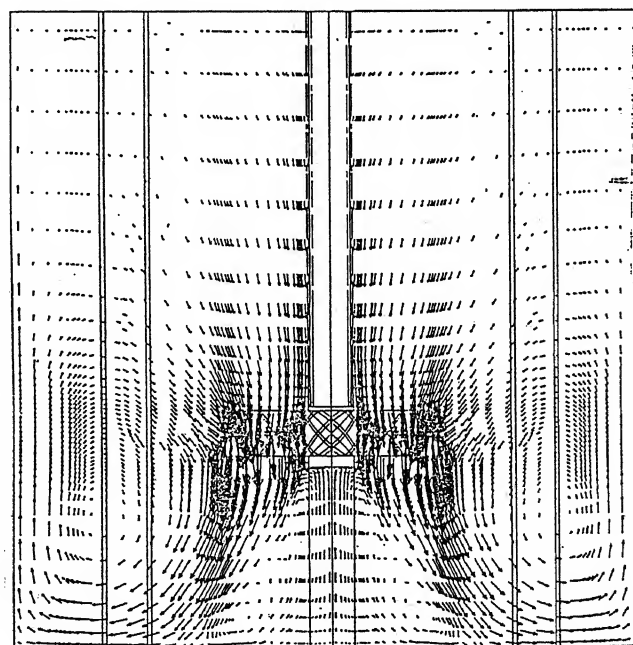


Figure 1. Stirred vessels (from ref. 19). Predicted vector plot at midplane between the baffles $D = H = 0.3$ m, downflow, pitched blade turbine, turbulent regime. D , vessel diameter, H , height of the reactor.

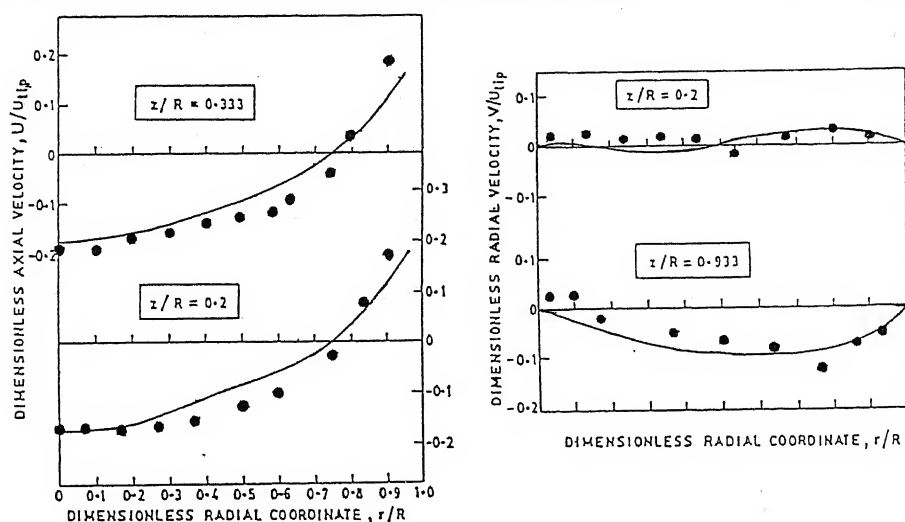


Figure 2. Stirred vessels (from ref. 20). Comparison of predicted mean velocity profiles with experimental data $D = H = 0.3$ m, disc turbine, turbulent regime.

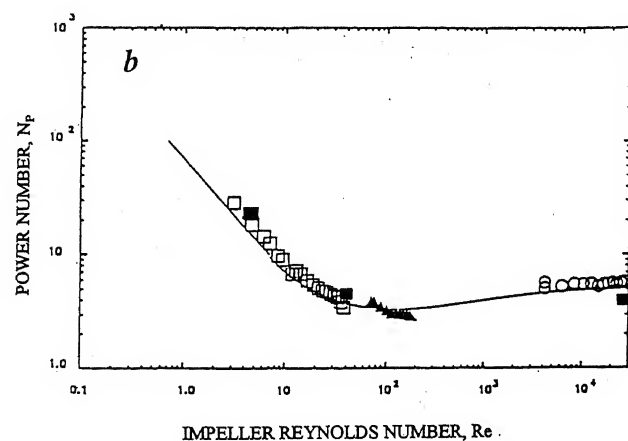
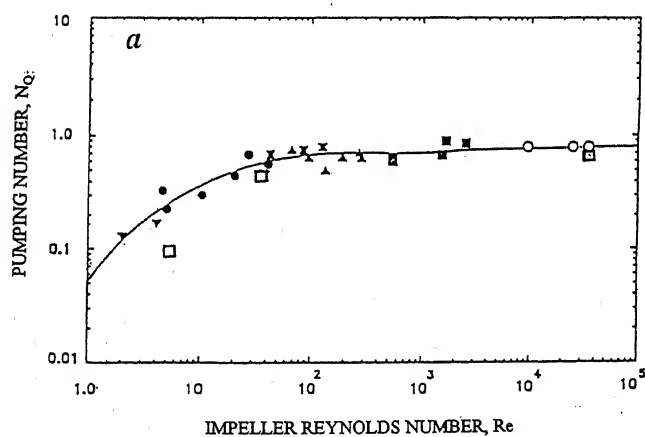
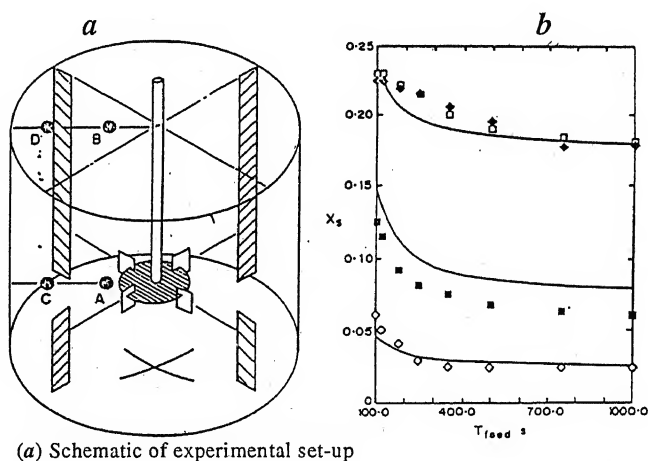


Figure 3. Stirred vessels (from ref. 20). Comparison of overall impeller characteristics with experimental data $D = H = 0.3$ m, disc turbine, turbulent regime.



(a) Schematic of experimental set-up

$T = 0.3$ m and 0.45 m
 $H = T$, $H_c = T/3$, $D = T/3$ Impeller – Rushton turbine

Feed point	r/R	z/R
A	0.368	0.0
B	0.368	1.22
C	0.690	0.0
D	0.690	1.22

H_c , Clearance of the impeller from the vessel bottom

(b) Comparison of different feed locations

$V_A = 0.019$ m³, $C_{B0} = 11.82$ mol/m³, $N = 156$ r/s,
 $N_{A0}/N_{B0} = 1.1$, $V_B/V_A = 0.01$

Symbol	Description
◇	Feed point A
■	Feed point B
◆	Feed point C
□	Feed point D
—	model

N , Impeller rotation speed.

Figure 4. Reactive mixing in stirred vessels (from ref. 22).

large parameter space, conventional design procedures rely on thumb rules and empirical correlations. New and innovative designs are often sidelined because of time and resource constraints on carrying out experimental studies. Flow modelling will prove to be an ideal tool to understand the fluid dynamics and performance of these mixers. The validated flow models can be used to screen the large parameter space to identify most promising configurations. The complex interactions of baffles, internals (like coils/spargers), shape of the vessel and impellers can be conveniently studied using the flow model.

We have developed a computational model, based on quasi-steady state approximation, to simulate flow generated by a variety of impellers in fully baffled stirred mixers^{19,20}. The vector plot of the predicted flow field generated by a downflow pitched blade turbine (PTD) is shown in Figure 1 for a typical r - z plane. This figure shows the well documented flow pattern of PTD. It should be noted that these results were obtained without using any empirical impeller boundary conditions. The model predictions also show satisfactory quantitative agreement with the experimental data (Figures 2 and 3) over the wide range of impeller Reynold number. The computations also revealed a wealth of details about the flow structure around the rotating impeller blades which are hard to obtain experimentally. The flow model adequately captures the influence of impeller location, blade width, blade angle, etc. on the generated flow and mixing. Other important details like interaction of baffles and rotating impellers, fluid forces on the impeller blades can be analysed using the flow model. The predicted flow results will therefore be useful for analysing and improving the blending, solids suspension, residence time distribution and scale-up performance of the mixers.

Enhancing selectivity of series-parallel reaction system in stirred reactor

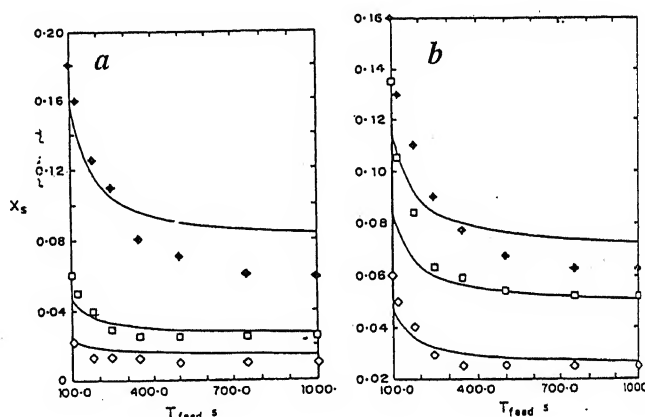
Many high-value chemicals (pharmaceutical products) are manufactured in stirred semi-batch reactors, in which the limiting reactant is added continuously to maximize the yield of the desired product. If the chemical reactions are fast, the yield and selectivity is mainly a function of mixing²¹. A variety of design parameters such as feed pipe location, feed pipe, impeller type and location, etc. affect the overall performance of such reactors. A flow model including the reactive mixing will be a very effective tool to optimize these systems.

We have considered a series-parallel reaction scheme carried out in a semi-batch reactor (schematic diagram of the considered reactor set-up is shown in Figure 4 a). A model was developed to understand the interaction of turbulent mixing and fast chemical reactions²². This re-

active mixing model was integrated with the flow model of the stirred reactor (as described earlier). The overall model was successfully applied to predict the yield and selectivity patterns of series-parallel reaction schemes under a wide range of parameters. The comparison of computed results with experimental data is shown in Figures 4 and 5. The model was used to evaluate the influence of feed pipe locations, stirrer speeds, reactant concentrations, reactor size and feed time, etc. on reactor performance. The study resulted in identification of optimum feed pipe location and feed rate of limiting reactants.

Bubble column reactors

A bubble column reactor (in which reactant gas itself provides the required stirring and mixing) offers an attractive way to carry out gas-liquid reactions because of its simple construction and easy operation. However, because of their simple constructions, bubble column reactors also have an inherent limitation of having fewer



a, Influence of stirrer speed on X_s for feed point A. Conditions are same as in Figure 4 except stirrer speed.

Symbol	Impeller speed, r/s
◇	3.78
□	2.60
◆	1.26
—	model

X_s , Yield of undesired product.

b, Influence of reactant concentration on X_s for feed point A. Conditions are same as in Figure 4 except reactant concentration.

Symbol	Concentration of B, C_{B0} , mol/m ³
◆	35.46
□	23.64
◇	11.82
—	model

Figure 5. Reactive mixing in stirred vessels (from ref. 22).

degrees of freedom available to control their performance characteristics. In bubble columns, local flow, turbulence and gas hold-up distribution are interrelated in a complex way with the operating and design variables. A detailed flow model therefore becomes a necessity to understand these complex interactions and to interpret the experimental results. One of the major disadvantages of bubble column reactors is the high degree of back-mixing prevailing in such reactors. Performance of these reactors can be substantially improved by appropriately tuning the degree of backmixing by employing suitable column internals like radial baffles. Flow models can be advantageously used to minimize the required experiments for identifying suitable internals and for confident scale-up with these internals.

We have developed²³ computational models to make quantitative predictions of turbulent fluid dynamics of bubble column reactors operated in a heterogeneous regime. Two submodels are proposed to account for the influence of bubble wakes and column walls on the motion of bubbles. Model predictions show satisfactory agreement with the published experimental data over the wide range of column diameter and superficial gas velocities. Figures 6 and 7 show the comparison of the predicted results with the experimental data obtained for bubble column of 0.29 m diameter for two different gas velocities. The influence of radial baffles on mixing in bubble columns was adequately represented by the computational model²⁴. Detailed three-dimensional predictions along with the submodel for the sparger will provide invaluable information for scale-up and fine tuning of large bubble column reactors.

Fixed bed reactors

Performance of fixed bed reactors crucially depends on the distribution of reactants. Deflection baffles are often employed to evenly distribute the feed vapours coming from the reactor inlet in the form of high velocity jets. A computational model was developed to optimize the design of deflection baffles for an industrial fixed bed reactor. Pressure losses can be accurately predicted. The reaction kinetics can be coupled with the flow model to predict the detailed temperature and concentration fields within the fixed bed reactors. Fixed bed reactors are also used in the radial flow mode for a variety of large scale processes (isomerization of *p*-xylene, ammonia synthesis, etc.). Fluid dynamics of radial flow fixed bed reactors (RFR) is very complex and involves abrupt changes in flow directions. Flow models can help to optimize the shroud and support screen design, to minimize the flow mal-distribution and to enhance the performance of the radial flow reactor. Such a study is recently reported by Ranade²⁵. The predicted flow field for three different conditions is shown in Figure 8. The corresponding ve-

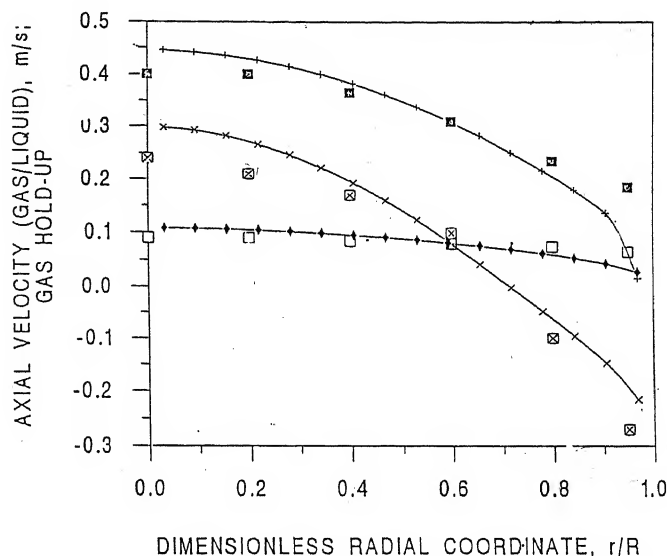


Figure 6. Bubble columns (from ref. 23). Comparison of predicted axial velocities of gas and liquid phase and gas hold-up with experimental data. $D = 0.29$ m, $H = 4.5$ m, gas velocity = 0.02 m/s.

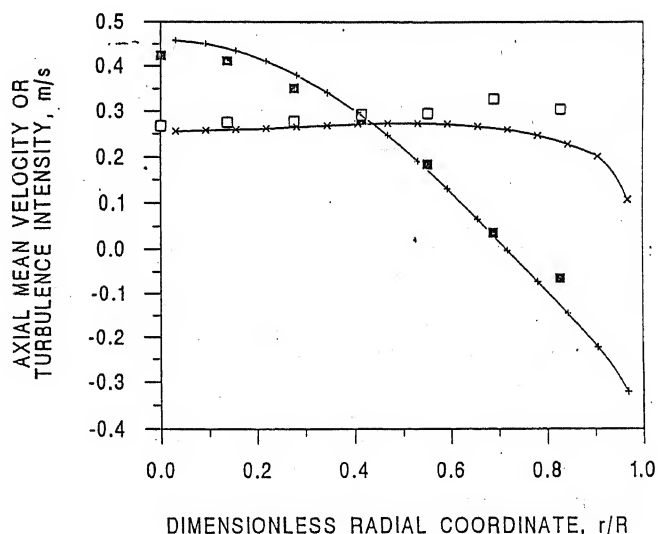


Figure 7. Bubble columns (from ref. 23). Comparison of predicted axial velocity and turbulence intensity with experimental data. $D = 0.29$ m, $H = 4.5$ m, gas velocity = 0.06 m/s.

locity profiles at the outlet of the catalyst bed are shown in Figure 9. It can be seen that, unless support screens are appropriately redesigned, loading more catalyst in the reactor (by filling zones B and C) may not lead to the desired increase in the throughput of the reactor (because of the severe mal-distribution evident from the profile *b* of Figure 9). Detailed flow modelling and simulations are therefore necessary to realize the full potential of the available catalyst (and to develop better understanding and insight).

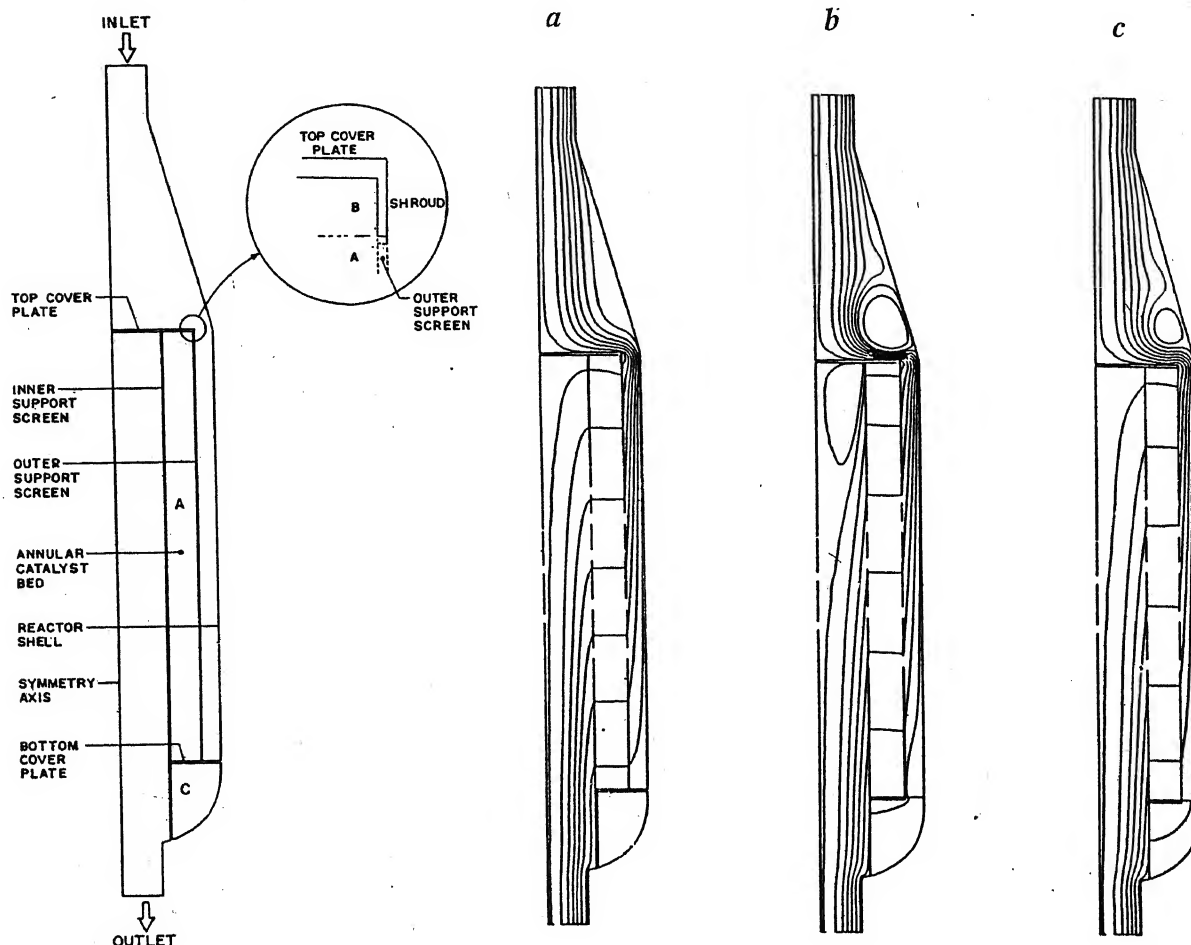


Figure 8. Radial Flow Reactor (RFR) (from ref. 25). Contour plots of stream function. Comparison of predicted flow field for three different configurations of RFR. *a*, Catalyst in zone A, top cover plate with shroud; *b*, Catalyst in zones A, B and C, no shroud, with screens same as in *a*; *c*, Catalyst in zones A, B and C, no shroud, with adjusted screen resistances.

The list of applications of flow modelling may be enlarged to encompass almost all types of process equipments. We have carried out flow modelling of jet mixers²⁶, flow meters²⁷, polymerization reactors, etc.

Industrial flow modelling: The Indian scenario

The opportunities and potential for enhancing performance of the existing assets of Indian process industries are truly enormous. Apart from improving the utility of existing assets, flow modelling tools offer a unique opportunity to bridge the gap between technology suppliers and technology buyers. The expertise built over the years of experimentation can now be developed within short span with substantially less resources by judiciously using flow modelling tools and expertise. Successful realization of these potential improvements

depends on several factors. Two of the most crucial factors are availability of flow modelling expertise to solve practical problems and willingness and commitment of Indian industries to upgrade their technologies by investing in indigenous flow modelling research.

Basic flow modelling requires expertise in fluid mechanics and numerical techniques. However successful modelling of industrial flow processes requires much more knowledge and expertise than just these two fields. More often than not, industrial flow problems cannot be modelled rigorously. Broad-based chemical and process engineering expertise along with the knowledge pool on different fields ranging from estimation of physical properties, rheology to kinetics and catalysis is therefore essential. New approaches of flow modelling need to be developed to make the complex industrial problems tractable without jeopardizing the objectives. We have made a small beginning at National Chemical Laboratory (NCL) by initiating an 'industrial flow modelling

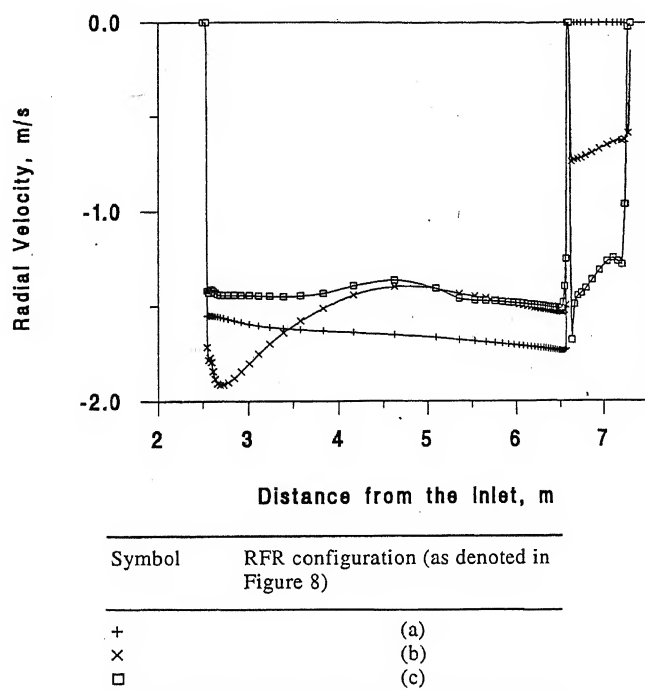


Figure 9. Radial Flow Reactor (from ref. 25). Profiles of radial velocity at the bed outlet.

group (iFMg)¹. We have an advantage of availability of a large chemical and process engineering knowledge pool at NCL. We are working on a variety of performance enhancement projects sponsored by Indian as well as multinational companies from abroad.

It is, however, essential to form a critical mass of industrial flow modellers (from research laboratories and industries) in order to derive the maximum benefits. Many industries in India have purchased commercial flow modelling codes. However, as far as we know, the use of these codes to solve real life industrial problems is very limited. In order to increase the level of confidence in the flow modelling approach, it is necessary to have more interaction between flow modellers and engineers working on practical problems. It may be necessary to form CFD user groups or to organize workshops to facilitate such interaction. (In October 1995, we had carried out one such workshop, along with the applications group of C-DAC, on industrial flow modelling.) This will help us to address the crucial question of availability of the required flow modelling expertise.

The other key factor of willingness of Indian industries to invest in flow modelling research also needs to be discussed here. Complex industrial flow problems cannot be solved in a few days. In the initial stages, it is indeed necessary to painstakingly build the required expertise to solve industrial flow problems either with indigenous or commercial CFD codes. Indian industry should, therefore, be willing to invest funds to develop

such an expertise and computing facilities for realizing the potential performance enhancements. Considering the costs involved (of manpower, hardware, software and other facilities), it may be worthwhile to formulate a consortium of industries (private as well as public sector) to fund such research on flow modelling of industrial processes.

It must be added here that first rate work on modelling of industrial processes can be sustained only if it is backed up by basic research in complex flows. It is necessary to identify short and long term goals for further research on both computational as well as physical aspects of industrial flow modelling. The most important areas of computational character in which further work is needed are:

- ways of conducting fine-grid computations cheaply;
- minimizing numerical diffusion without jeopardizing the robustness;
- preserving order and flexibility in CFD codes as the complexity of their physical content increases.

Further research on problems of physical character primarily concerns the development of better turbulence models and formulation of tractable approaches for simulating industrial flows involving inherently unsteady large scale flow structures. Recent advances in applying renormalization group (RNG) theory need to be explored further. The consortium mentioned earlier should therefore also invest part of their funds for such basic research. Such investments on flow modelling research will help us to bridge the gap which separates us and technologically advanced nations.

Concluding remarks

In this review, we have described the overall methodology for modelling of industrial flow processes. The opportunities and potential of using flow modelling techniques for realizing performance enhancement were discussed. The basic framework of computational fluid dynamics was described. Application of the suggested methodology was illustrated with the help of case studies.

Predictions of CFD models can go wrong for two main reasons (other than human error and machine malfunction): they may be based on physically incorrect mathematical representations of basic physical phenomena or upon numerically deficient representation (incorrect discretization, inadequate resolution, incomplete convergence and so on). The inadequacies of the later kind are easier to quantify. The bounds of numerical accuracy of the specific code can be obtained by comparing results with the analytical solution of some simple but representative problem. Villasenor and

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Spalding²⁸ have presented 14 flow problems which can be used for the benchmarking exercise. Recently Haworth *et al.*²⁹ proposed a useful approach to error estimation and physical diagnostics in multidimensional CFD simulations. Only a carefully planned validation and benchmarking exercise can transform the CFD model and code into a useful design tool. Inadequacies of the mathematical model are almost always present because the physics of complex, turbulent and multiphase flows is not yet properly understood. It is the duty of flow modeller to identify those aspects of flow, which are crucial to process performance and represent it with adequate accuracy in the mathematical model.

Despite their limitations, computational fluid dynamic models have been shown to be capable of predicting detailed flow fields within industrial process equipment. CFD models can also be used to study aspects of flow which are not easily amenable to experiments (e.g. high temperature). This unique capability of a computational tool may have most impact on equipment engineering practice. Detailed flow modelling of industrial processes offers new possibilities for performance enhancement and innovation in the design of process equipments. With the emergence of cheap, high speed computing platforms and availability of the commercial CFD codes and support, flow modelling needs to be properly exploited by the process industry to maximize the performance of the various processes and process equipment.

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Transforming growth factor beta (TGF- β) in bone remodelling

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The current concepts of the role of transforming growth-factor beta (TGF- β) with regard to bone metabolism and fracture repair are reviewed. The sources, types, chemistry, cellular activity and the clinical applications of TGF- β have been discussed. In low concentrations, this growth factor has significant effects on bone remodelling. The use of TGF- β for practical therapeutic purpose remains an exciting challenge.

BONE differs from other tissues not only in physiochemical structure but also in its extraordinary capacity for growth, continuous internal remodelling and regeneration throughout postfetal life. Bone formation is a complex process regulated by diet, vitamins, hormones and growth factors¹. Growth factors are polypeptides that increase cell replication and have important effects on differentiated cell function. Growth factors were initially considered as systemic agents, but current evidence indicates that they act primarily as local regulators of cell growth². The transforming growth factor- β family of proteins, which comprises polypeptide growth factors that have diverse effects on the growth, differentiation, and function of cells, is receiving considerable attention for potential clinical applications and is likely of physiological and surgical significance.

Transforming growth factors

It is one of the most important growth-promoting osteoinductive substances, which have been identified at the site of fractures and other places. Transforming growth factors (TGFs) have been categorized into TGF- α (alpha) and TGF- β (beta). TGF- α has not been isolated from bone tissue and cannot be considered a local regulator of bone remodelling, although it is mitogenic for bone cells and stimulates bone resorption. TGF- α shared amino acid sequence, receptor-binding and functional activity with epidermal growth factors but had only weak transforming growth factors activity³. Furthermore, TGF- β by itself was completely ineffective, but together with TGF- α it potently induced growth of colonies of cell-line³.

TGF- β is a multifunctional growth factor that has been shown to mediate normal cellular physiology and tissue

embryogenesis and to participate in a variety of responses associated with inflammation and tissue repair⁴.

Transforming growth factor-beta

Sources

TGF- β was originally purified from human platelets⁵, human placenta⁶ and bovine kidney⁷. The largest source of TGF- β in the body is the extracellular matrix of bone and platelets may represent the second largest reservoir for the peptide⁸.

Chemistry and types

TGF- β is a highly stable molecule that consists of two identical chains, each containing 112 amino acids. TGF- β s are a family of polypeptide growth factors encoded by closely related genes. There are at least five TGF- β s: TGF- β_1 to TGF- β_5 . TGF- β_1 , β_2 and β_3 have been found in many species, including humans; β_4 has been found in chickens and β_5 has been found in amphibians⁹. All share 64–82% similarity in their amino acid sequence¹⁰. Human platelets contain a single form of TGF- β composed of two identical polypeptides constituting a dimer of relative molecular mass (Mr) 25,000 (ref. 11).

A very high level (more than 95%) of sequence homology for a single isoform of transforming growth factor- β among many species indicates that these five isoforms are not simply species-specific variants¹². All the five isoforms might be expressed within a single species, in which all or a subset of the transforming growth factors β s may be synthesized by particular tissue at specific stages of development, or, after appropriate stimulation. A number of biochemicals featured are shared by most of these closely-related proteins⁴. The mature TGF- β subunit contains two identical chains, each containing 112 amino-acids (except TGF- β_4) and 9 cysteine residues whose locations are conserved in all five isoforms¹³. The active proteins are approximately 25 kDa and are composed of two, usually identical, disulphide-linked subunits¹⁴. Physical characteristics of the mature polypeptide subunits of TGF- β s are listed in Table 1.

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Table 1. Physical characteristics of the mature polypeptide subunits of TGF- β s¹⁵

Protein	Amino acids per subunit	Molecular weight (Mr) (Da)	Conserved cysteine
TGF- β_1	112	12,500	9
TGF- β_2	112	12,500	9
TGF- β_3	112	12,500	9
TGF- β_4	114	12,900	9
TGF- β_5	112	12,500	9

TGF- β is synthesized in precursor form that requires proteolytic processing. It is released from the cell in an inactive high molecular weight complex composed of the processed dimer in combination with amino terminal fragments of each of the two TGF- β monomeric precursors and a third uncharacterized molecule. The physiological mechanism by which TGF- β is released from the inactive complex is uncertain but may require specific enzyme activation^{16,17}.

Cellular activity

Transforming growth factor- β has a broad range of cellular activities, including the control of the proliferation and expression of the differentiated phenotype of several types of cells specific to the skeleton, among them the pluripotent undifferentiated mesenchymal cells (pericytes) for chondrocytes, osteoblasts and osteoclasts¹⁸. *In vivo* and *in vitro* studies have demonstrated the synthesis of TGF- β by chondrocytes and osteoblasts and their expression is increased in fracture callus and specifically regulates bone-associated activities that may be important for the initiation of repair of fracture¹⁹. Studies on the potential role of transforming growth factor β in soft tissue wound healing have shown that this molecule is released from degranulating platelets at the site of an injury, possibly to initiate a cascade of reparative events²⁰.

Histologically, TGF- β increases nuclear 34-thymidine labelling in the osteoblast precursor cell zone²¹. Studies with isolated bone cells show that TGF- β is a potent regulator of osteoblastic cell activity and on a molar basis TGF- β is one of the most effective mitogens so far described for osteoblast-enriched cultures from fetal bone^{22,23}.

Studies have also shown that TGF- β is the most potent chemotactic factor released from macrophages⁸. In addition to attracting macrophages to sites of injury, this peptide activates the synthesis of other growth factors and deactivates the production of hydrogen peroxide by macrophages, so that young tissues are not destroyed²⁴. Moreover, TGF- β has been shown to stimulate fibroblast-directed tissue repair by upregulating the production of extracellular matrix components, such as

collagen, fibronectin and proteoglycan, by upregulating the expression of cellular integrin receptors for extracellular matrix proteins; and by inhibiting the action of proteolytic enzymes that could destroy newly-formed osseous tissue¹⁶.

TGF- β produce pluripotent and biphasic changes in the biochemical activities of cells at particular stages of differentiation within the osteoblast lineage⁴. To date, most studies to evaluate the effectiveness of TGF- β s in bone are performed with the prototypical isoform of TGF- β_1 derived from blood platelets, but TGF- β_1 , β_2 , β_3 each have identical qualitative effect in osteoblast enriched cell cultures²².

All the three isoforms potently increase synthesis of bone DNA at low concentration but have reduced mitogenic activity at higher levels⁴. These isoforms also enhance synthesis of collagen and non-collagen protein and decrease activity of alkaline phosphate in osteoblast enriched cultures, but TGF- β_3 appears to be 3–10 times more potent than TGF- β_1 and β_2 (ref. 25). Similarly, the TGF- β s enhance replication of cells and production of bone matrix in cultures of intact bone *in vitro*. In most instances, the TGF- β s enhance synthesis of type-1 collagen and non-collagen protein in bone cells²⁶. The mitotic response to TGF- β is biphasic; low concentrations (below 100 rM) are stimulatory, whereas higher levels produce less of a stimulatory effect. At higher, less mitogenic concentrations, TGF- β alters expression of various activities associated with the osteoblast phenotype in different ways; under these conditions, TGF- β decreases alkaline phosphatase activity and similar to its effects in a number of other connective tissue systems, enhances the synthesis of type I collagen, the major organic element in the bone matrix^{22,27}. Furthermore, TGF- β_1 and β_2 enhance chemotaxis of osteoblasts²⁸, suggesting an additional role involving the recruitment of differentiated osteoblasts that may be necessary for new bone formation and fracture repair.

Recent studies in osteoblast-enriched cultures have demonstrated that TGF- β_1 increases synthesis of type 1 collagen polypeptide in the absence of mRNA transcription and decreases non-collagen in the bone matrix as a result of the action of TGF- β probably occurs by multiple transcriptional, post-transcriptional and post-translational events⁴. Some other activities of TGF- β s associated with the function of osteoblasts have also been examined. TGF- β_1 and β_2 increase the transcriptional and polypeptide levels of osteopontin, a protein in the bone matrix that is thought to be important in adhesiveness of bone cells²⁹. In osteosarcoma cultures, TGF- β enhances Type 1 collagen synthesis as well as the production of a variety of bone matrix-associated polypeptides such as osteonectin, osteopontin and alkaline phosphatase^{29–33}, but decreases osteocalcin synthesis³⁴. These findings indicate that TGF- β enhanced bone matrix accumulation and consequently the maintenance of

bone mass. In general, the stimulatory influence of TGF- β on bone matrix protein synthesis may be attributed to effect at the transcriptional level^{22,27,31}.

However, a direct relationship between the influence of TGF- β on type I collagen mRNA and polypeptide levels is not observed in isolated bone cells, suggesting that TGF- β has an additional supportive role in increasing bone matrix formation²². The protein in the bone matrix termed osteonectin, which may be involved in the deposition of type-I collagen and the transition between cartilage and bone, was increased by TGF- β_1 , in an *in vitro* model that was used to study repair of fracture³⁵.

Much less information is presently available regarding direct effects of the TGF- β s on osteoclasts or osteoclast precursors. Recently, however, biphasic effects on the development of osteoclast-like cells have been reported, in which low concentrations were stimulatory and higher concentrations were inhibitory. TGF- β_1 induces resorption of bone on synthesis of prostaglandin E₂ (ref. 36).

In fetal rat long bones, TGF- β decreases bone resorption³⁷, and this effect may be related to the ability of TGF- β to inhibit the formation of osteoclast-like cells *in vitro*³⁸. This difference may be a significant distinction in bone growth and development between the fetus and the new born; within very early stages of bone formation, matrix formation may be more important to the organism than bone remodelling. It is therefore suggested that TGF- β has an additional, indirect role in increasing or maintaining body mass³⁷.

With regard to formation of cartilage, the TGF- β s appear to increase differentiation of mesenchymal cells, production of proteoglycans, and replication of chondroblasts³⁹. In contrast, these agents usually decrease the proliferation and function of differentiated cells by more mature chondrocytes. For example, TGF- β s decrease the activity of alkaline phosphatases²⁷. The highest levels of TGF- β mRNA were associated with osteoblasts in developing bone, whereas TGF- β transcripts decreased in chondroblasts/chondrocytes with increased type II collagen expression⁴⁰.

In general, it seems that one important role for the TGF- β s is to include developmental transitions in cells that are involved in formation of endochondral bone at particular stages of differentiation. The predominant trend of these effects appears to be toward eventual *de novo* formation of bone⁴.

Clinical applications

The use of TGF- β s to enhance wound healing was first investigated by Mustoe *et al.*⁴¹, who applied this peptide directly to and immediately after the creation of linear incisions through the dorsal skin of rats. These investigators demonstrated a 220% increase in the maximum strength at the site of the wound after five days and an

acceleration in the rate of healing by at least three days compared with that in the control animals.

It is also suggested that this peptide molecule has the capacity to stimulate both intramembranous and endochondral formation of bone³⁵. Noda and Camilliere⁴² studied the effect of daily injection of 1 μ g of TGF- β directly into the periosteum of the parietal bones of neonatal rats. They showed that (i) the thickness of the treated parietal bones increases approximately twofold, (ii) this effect was localized to the site of the injection, (iii) no changes were observed in the contralateral bones or at distant skeletal sites.

Joyce *et al.*¹⁹ performed a similar study in the femora of newly-born rats and found that on daily injection of either TGF- β_1 or β_2 into the subperiosteal region of the femora, mesenchymal precursor cells in the periosteum were stimulated to proliferate and differentiate much the same as is observed during the embryological formation of bone and early fracture healing. After cessation of the injection, endochondral ossification also occurred and this resulted in the replacement of cartilage with bone. In addition, it was shown that injection of TGF- β_2 stimulated the synthesis of TGF- β_1 in chondrocytes and osteoblasts, suggesting autoregulation of this peptide^{9,19}.

It has been demonstrated that a single injection of 25–100 nanogram (ng) of recombinant human TGF- β_1 adjacent to the ear cartilage of rabbits stimulated the formation of bone after 21 days⁴³. In a subsequent investigation, they showed that exogenously applied recombinant human TGF- β stimulated the recruitment and proliferation of osteoblasts in critically-sized defects in the skull of rabbits. By assessing the temporal dynamics of the formation of bone in these defects, they demonstrated the potent osteoinductive activity of this peptide and its potential therapeutic applications for non-healing osseous defects⁴⁴.

The function of TGF- β , both *in vitro* and *in vivo*, can be highly variable. In some instances this growth factor inhibits cell growth and matrix synthesis, whereas in others it stimulates these processes. These different functions depend on the target cell, the presence or absence of other cytokines and the dose⁴⁵. In their study, Sumner *et al.*⁴⁶ suggested that the response to treatment with TGF- β was dose dependent, with the lower dose being more effective for enhancing bone ingrowth, whereas, high dose of TGF- β inhibited mineralization. Synthesis of osteocalcin, a calcium binding protein important in mineralization⁴⁷ is inhibited, possibly due to this growth factor.

A similar observation was also reported by Nielsen *et al.*⁴⁸, who injected TGF- β , 4 to 40 ng in every alternate day for 40 days after creation of tibial fractures in rats. Mechanical testing showed that TGF- β induced a dose-dependent increase of the callus at the site of the fracture.

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Enhancement of bone ingrowth with TGF- β was also evaluated in the canine model⁴⁶. Sumner *et al.*⁴⁶ observed that treatment of an implant (titanium fibre metal-coated rod) with a combination of a hydroxyapatite-tricalcium phosphate coating and TGF- β may result in better bone ingrowth than that obtainable from grafting of the gap with autogenous cancellous bone. The amount of new bone formation in the three millimeter gaps adjacent to the treated implants was twice that in the gaps of the paired controls, regardless of the dose. The differences between the treated and control implants with regard to architecture of the new bone in the gap indicate that the mechanism of action of TGF- β_1 may include both proliferation of osteoprogenitor cells and production of matrix by committed osteoblasts⁴⁶.

Histological study with two *in vivo* models of osteogenesis have also demonstrated an increase in net formation of bone by TGF- β_1 and β_2 , when the agents were tested either by direct application to the tissue or by subcutaneous injection⁴².

Summary

The transforming growth factor- β s are polypeptide growth factors encoded by a family of closely-related genes that are expressed in numerous tissues and species. Bone was one of the first tissues in which locally-produced molecules with TGF- β like activity appeared to regulate normal cellular function, and the skeletal matrix probably comprises the largest reservoir of TGF- β s. *In vitro* and *in vivo* studies have indicated that TGF- β s can have either stimulatory or inhibitory effects on replication, lineage development, and differentiated phenotypic function in many types of skeletal tissue cells⁴.

The effect may be biphasic in the sense that low concentrations of TGF- β s stimulate proliferations of cells, whereas high concentrations inhibit proliferation. Even within the same cell lineage, opposite effects have been noted with different concentrations of TGF- β s, or with skeletal cells at different development stages⁴.

In foetal tissue, TGF- β stimulates differentiation of mesenchymal cells, proliferation of osteoblasts and synthesis of bone matrix, but it may also induce maturation into and through the osteoblast, chondrocytes and osteoclast lineages⁴.

Most evidence to date leads to the conclusion that the TGF- β s have an important role in the formation of bone and this has tremendous potential in therapeutic application for non-healing osseous defects and in different types of fracture healing.

Finally, continued *in vivo* and *in vitro* studies of these molecules are needed in order to evaluate their effective doses, appropriate duration of treatment, tissue specificity, receptor-binding, intracellular signalling and the

regulation of their activities by other local and systemic factors.

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RESEARCH ACCOUNT

Bile acids in asymmetric synthesis and molecular recognition

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This account summarizes the progress made in our laboratory towards the development of new uses of naturally occurring bile acids. Applications in Asymmetric Synthesis (intramolecular coupling, and intermolecular reactions) and Molecular Recognition are described with suitable examples.

NATURE is asymmetric and almost all chiral molecules present in nature are homochiral (i.e. they exist in only one of the two possible enantiomeric forms). Organic chemists have always been fascinated by the possibility of constructing natural products by total synthesis. This approach was very successful till the sixties for constructing racemic modifications of chiral natural products. The methodology for synthesizing only one (the naturally occurring form) enantiomer was not present in the organic chemists' 'toolbox' until the seventies. During the past two decades, however, asymmetric synthesis of a variety of molecules has been accomplished by chemists using an assortment of techniques. In most of these examples, the inherent chirality of natural products (such as terpenes, sugars, amino acids etc.) has played important role.

The 'lock and key' concept of Emil Fischer for explaining the specificity of enzymatic action has been known for a century. Deliberate attempts to mimic such biological processes in the laboratory with small organic molecules, however, started much later. Early work with cyclodextrins and subsequently with crown ethers were forerunner to a new area of research towards the design, synthesis, evaluation and applications of synthetic molecular receptors. Carefully designed studies on molecular receptors have provided new insight into molecular interactions. In addition, recent research has shown that many molecular receptors can be designed to have tailor-made properties and hence can be used as a variety of molecular devices including molecular sensors (see, for example, ref. 15).

1. Bile acids: their properties

Bile acids, secreted by the liver, are important metabolites of cholesterol (Figure 1). These compounds are sometimes found in the form of conjugates, specially with glycine and taurine. Most bile acids are

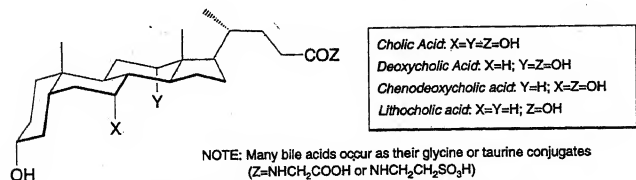


Figure 1. Representative examples of bile acids.

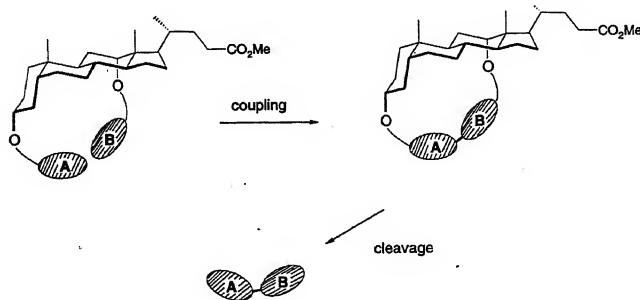


Figure 2. Schematic representation of the bile acid unit acting as a template for a coupling reaction.

characterized by two distinct faces, one being the hydrophobic top (β) face, while the other (α , bottom) face is functionalized with polar hydroxyl groups. In addition, the side chain terminates in a carboxylic acid or other polar functionality. These acids are believed to aid in fat metabolism *in vivo*¹.

A great deal of work has been carried out during the past three decades on the inclusion properties of bile acid crystals². The crystals of cholic and deoxycholic acids contain channels, wherein guest molecules such as low molecular weight ketones can be trapped.

We were intrigued by the orientation of the hydroxyl groups of varying reactivity arranged on the α -face of the bile acid backbone. Until the mid-eighties, there were no reports on the use of the bile acid moiety towards the design of chiral auxiliaries and molecular receptors. We set out to develop new chemistry of bile acids, and use them for asymmetric synthesis and for the design of novel molecular receptors. The progress we have made in the past six years is summarized in this account.

2. Bile acids in asymmetric synthesis

Since the late seventies, there has been considerable progress towards achieving asymmetric synthesis of bioactive molecules³. Numerous chiral auxiliaries and catalysts, such as those derived from amino acids, terpenes, sugars, etc. have been utilized. Surprisingly, readily available optically pure bile acids did not receive any attention at all.

We have used bile acids in two distinct ways to induce chirality in reaction products. One approach, described in §2.1, was explored for *intramolecular* coupling proc-

esses. The other strategy (§2.2) made use of cholic acid as a chiral auxiliary for *intermolecular* reaction.

2.1. Bile acids as templates for intramolecular coupling

7-Deoxycholic acid, shown in Figure 1, has two hydroxyl groups whose chemical reactivities are different. We decided to examine the possibility of attaching two reactive units to the two hydroxyl groups, and couple them to make one or more bonds. Since the bile acid moiety is chiral, it was anticipated that if the coupling generates one or more stereogenic centers, the product might be formed stereoselectively. This concept is shown schematically in Figure 2.

Our first attempt involved the coupling of two 4-substituted aniline fragments covalently attached to the chiral bile acid template. The reaction of 4-toluidine with formaldehyde and hydrochloric acid was examined more than a century ago by Julius Tröger⁴. The product from this reaction (Tröger's base 1), and its derivatives, have recently attracted a lot of attention for their rigid V-shaped structure which allows one to use this structural unit as a scaffold for the design of molecular receptors^{5,6}. We have demonstrated that with a suitable precursor (2 or 3), the template coupling does indeed occur efficiently⁷, with diastereoselectivity approaching 75% (3 producing an excess of 5a) and with good chemical yield (Figure 3). The stereochemistry of the newly-formed stereogenic centers in the major product was unambiguously determined by a combination of chemical and spectroscopic means, including the X-ray crystallographic analysis of a single crystal (compound 4a)^{8,9}.

More recently, we have shown that a similar strategy can also be employed for the asymmetric coupling of two 2-naphthol fragments (6 to 7) on the template (Figure 4)¹⁰. Chiral binaphthols are increasingly being used for carrying out a variety of catalytic asymmetric transformations, and further developmental work might lead to a *practical* template-based asymmetric synthesis of this versatile chiral unit.

2.2. Bile acids as chiral auxiliaries for intermolecular reactions

A slightly modified approach was adopted by us for using cholic acid as a chiral auxiliary for *intermolecular* reactions. We reasoned that we could make use of the 3-position of cholic acid for tethering a reactive unit, and the 7-hydroxyl group to position a flat aromatic surface for *shielding* one face of the reactive group. The target molecule (acrylate ester 8) was synthesized readily in 7 steps from cholic acid in good overall yield. We found that the Diels-Alder reaction of 8 with cyclopentadiene at low temperature in the presence of a Lewis acid oc-

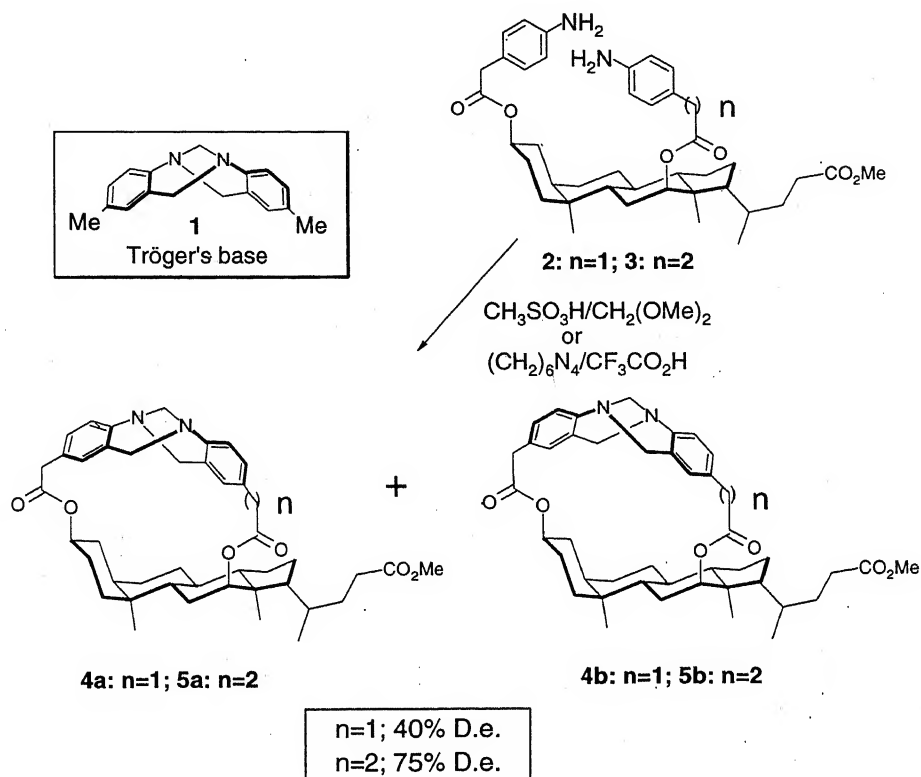


Figure 3. Template synthesis of Tröger's base derivatives on a bile acid template.

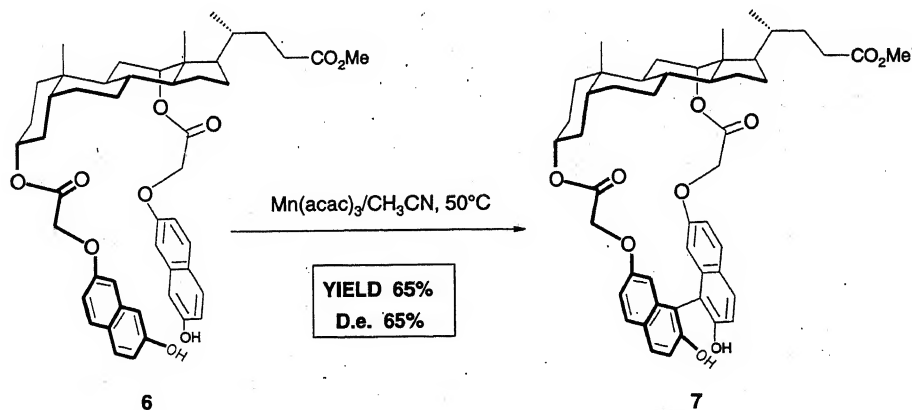


Figure 4. Template synthesis of a binaphthol unit on a bile acid template.

curred readily to give the adduct in very high yield and with diastereoselectivity approaching 90% (**9a** in excess, Figure 5). That the naphthalene ring was indeed functioning as anticipated was confirmed when the 3-acrylate ester containing an acetate at C-7 (instead of the naphthalene as in **8**) failed to show any stereoselectivity under identical conditions. The Diels–Alder product was removed from the adduct by an iodolactonization procedure, regenerating the chiral auxiliary in 88% yield^{11,12}.

The same strategy has also been shown to be moderately effective for the stereoselective reduction of 2-ketoesters as well. The steroidal chiral auxiliary was attached this time to pyruvic and phenylglyoxalic acids to produce **10** and **11**, respectively, and low temperature reduction with lithium borohydride resulted in 70% stereoselectivity in the diastereomeric product mixture (**12** and **13** were major products, Figure 6). The absolute configurations were assigned by comparison of spectral

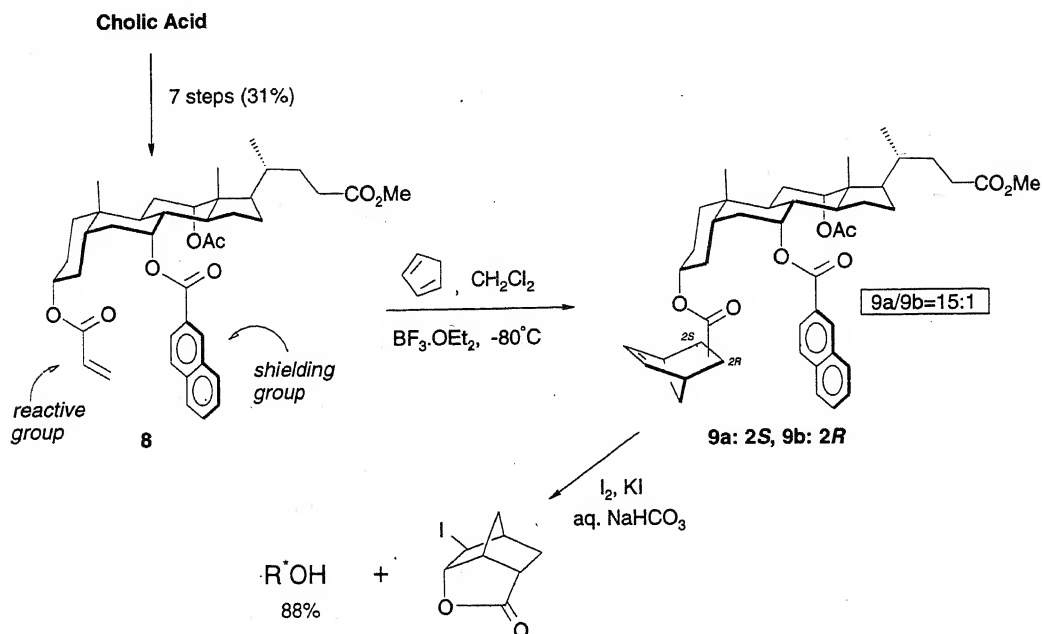
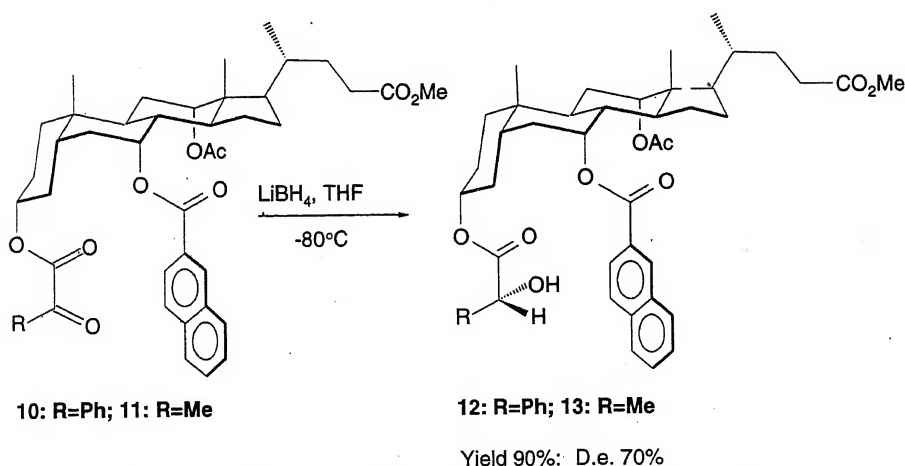


Figure 5. A cholic acid-based chiral auxiliary for asymmetric Diels–Alder reaction.

Figure 6. Asymmetric reduction of an α -ketoester on a steroidal auxiliary.

data with those of authentic samples¹³. Efforts to improve the diastereoselectivity are currently in progress in our laboratory.

3. Bile acids in molecular recognition

During the past two decades, there has been an explosive growth in research in the general area of 'Molecular Recognition' with synthetic receptors. A variety of molecular hosts have been constructed and their properties studied¹⁴. A number of potential applications of such designer molecules, including their use as

sensors¹⁵, can be envisaged. At the time our work was initiated, not much attention was focused on the use of bile acids for the design of molecular receptors. During the past five years, however, bile acids have been utilized by a number of groups for this purpose^{16–21}.

The unique arrangement of the three hydroxyl groups, along with the rigidity of the bile acid backbone, prompted us to construct novel molecular structures on the bile acid backbone capable of complexing small molecules or ions. The long term goal in this work is the development of molecular and ionic sensors, synthetic catalysts, novel organic materials, etc.

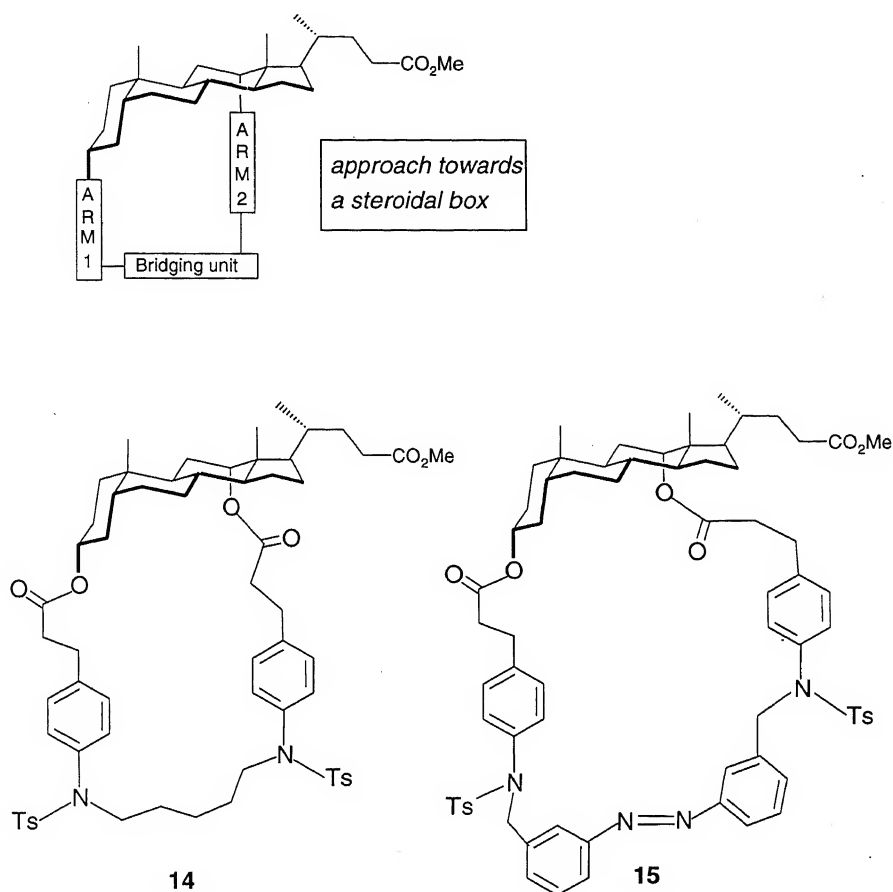


Figure 7. Molecular boxes on a deoxycholic acid scaffold.

3.1. Molecular boxes

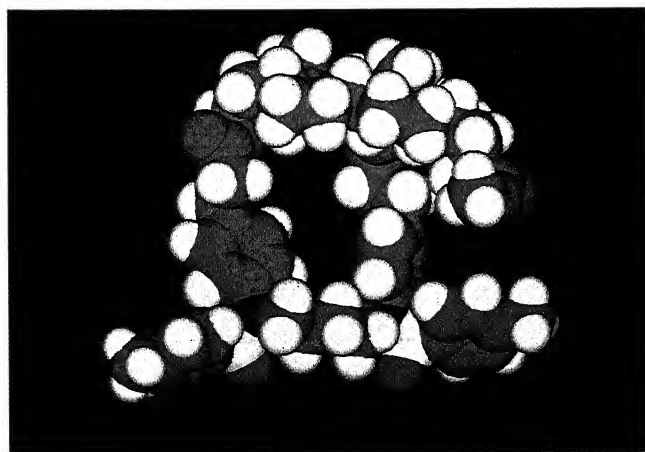
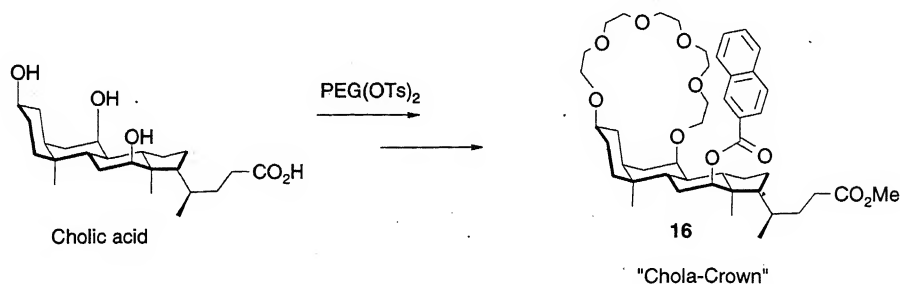


Figure 8. A space-filling representation of the INSIGHT II minimized structure of molecular box 14. The aromatic unit attached to C-3 (left) is blocking a part of the cavity.

A simple approach adopted by us is schematically shown in Figure 7, which utilizes the two OH-handles provided by 7-deoxycholic acid. It occurred to us that by attaching two molecular 'arms' to these two handles, and finally bridging them with a third linker unit, we will be able to generate bile acid-based molecular boxes. Even though synthetically it was feasible, as we demonstrated by efficiently constructing compounds **14** and **15** (ref. 22), these boxes surprisingly did not seem to have enough room in the interior to encapsulate any 'guest' which we examined. Recent calculations²³ suggest that the two arms which were attached to the bile acid tend to adopt conformations in which one of the aromatic rings protrudes inside the cavity, thereby blocking the entry of any guest (Figure 8). Nevertheless, we believe that further work along these lines with other more rigid arms will lead to cavities capable of encapsulation of molecular guests.



Association constants in CHCl_3 at 25°C

M^+	$\text{Log}K_a(\text{M}^{-1})$
Na^+	4.31
K^+	5.43
Rb^+	5.17
Cs^+	4.64
${}^t\text{BuNH}_3^+$	3.55

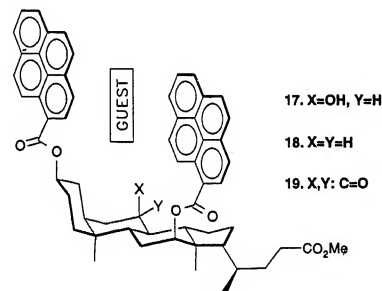
Figure 9. Cation binding with 'Chola-crown'.

3.2. Chola-crown

A variation of the molecular box approach which we examined was to build a crown ether by linking two of the three hydroxyl groups of cholic acid by a single pentaethylene glycol unit. Since cholic acid has three hydroxyl groups, we believed that a 'Chola-crown' having another available functional group (OH) will be a potentially useful system for the construction of metal ion sensors. Relative reactivity of the hydroxyl groups, and geometric considerations suggested that Chola-crown **16** shown in Figure 9 might be produced in *one* step from cholic acid. Indeed, this was found to be the case²⁴. The low yield (ca. 10%) in the synthesis was acceptable since both reactants (cholic acid and pentaethyleneglycol ditosylate) are readily available. Alkali metal ion binding studies have shown that this molecule shows a slight preference for K^+ over other cations. We believe that this system will provide opportunities for the construction of potential alkali metal ion sensors.

3.3. Molecular tweezer

A variety of tweezer-like molecules have been known, which are characterized by the positioning of converging binding sites available within a single molecule. In synthetic molecular tweezers, the binding interactions commonly employed are π -stacking and H-bonding interactions. Some recently-synthesized molecular tweezers have shown high (ca. 10^5 M^{-1}) binding affinities



Association constants (M^{-1}) in CDCl_3 at 25°C

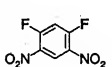
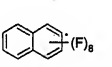
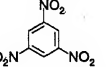
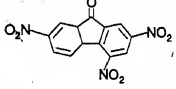
HOST				
17	6	11	47	147
18	7	15	65	165
19	8	19	83	224

Figure 10. Complexation of electron-deficient aromatic compounds with bile acid-based molecular tweezers.

towards 9-alkylated adenines²⁵. We felt that the functionalization of the 3- and the 12-positions of bile acids can lead to a new class of molecular tweezers. Unlike the synthesis of molecular tweezers constructed else-

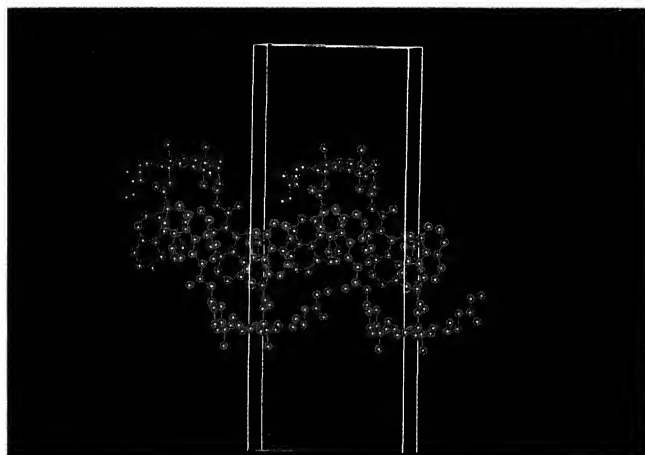


Figure 11. X-ray structure of compound 19: view of the packing diagram down the crystallographic *c* axis.

where, this approach is rather straightforward. Accordingly, we attached 1-pyrenoyl groups to these two positions of bile acids to construct a series of three steroidal molecular tweezers (17–19). Molecular modelling studies showed that a family of low energy conformations (gas phase) existed in which the two pyrene units could adopt approximately parallel orientations, thereby generating a deep cleft in between. Detailed NMR titration experiments showed that electron deficient aromatic compounds shown in Figure 10 bind moderately well to these hosts in chloroform²⁶.

The molecular structure of a single crystal of 19 has recently been solved²⁷. It is interesting to note that the conformation of the molecule is different from what we propose to be the 'binding conformation' in chloroform solution. The steroid molecules pack back to back, forming a bilayer, with the pyrene units arranged in a 'herringbone' fashion. One view of the crystal packing is shown in Figure 11.

More recently, we have immobilized the molecular tweezer to Merrified resin. Binding experiments by HPLC showed that the relative binding affinities of the immobilized host followed the pattern observed in solution. Further research in this area can possibly lead to the design of molecular filters.

Conclusions and future outlook

We have shown in this brief account how readily available bile acids can be utilized for a variety of purposes. We are currently exploring many other possibilities, such as the design of transition metal complexes using bile acid-based ligands, construction of bile acid-based

dendrimers, design of host libraries based on bile acids, etc. We believe that the outcome of some of this work may possibly lead to the development of molecules of considerable interest for practical applications.

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Molecular band polarization in Comet Hyakutake C/1996 B2

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IAU/IHW filter set is used to study the polarization in Comet Hyakutake in various molecular bands. The observations were taken on March 13.97 and March 18.88 UT dates using 1.2 m telescope at Mount Abu Observatory, India. The errors in the measurement of the percent polarization are very low ($<0.5\%$). The phase dependence of the polarization of C_2 and CN molecular emission bands due to resonance fluorescence is in good agreement with theory. However, the observed polarization in C_3 molecular band is found to be about 3% which is less than the predicted value. In the case of CN molecule, significant deviation is noticed in the direction of polarization vector from normal to the plane of scattering.

COMETARY polarimetry provides a very good technique to understand the properties of gas molecules and dust. The level of polarization is strongly correlated with the activity of nucleus and the presence of jets which exhibit increased polarization over the surrounding coma. The mechanisms for the cometary polarization are now known to be: (i) scattering of sunlight by the cometary particles, and (ii) the resonance fluorescence emission by the cometary molecules. However, the molecular

band polarization in visible spectral range has not been explored much, neither observationally nor theoretically. The theory of polarization of molecular bands was studied a long time ago by Mrozowski¹, though this work does not deal with molecular bands observed in comets. Since then there seems to be no theoretical work done on this subject. As far as observations are concerned, Ohman^{2,3} showed for the first time the presence of polarization in comets due to resonance fluorescence emission. Further works are by Blackwell and Willstrop⁴, Bappu *et al.*⁵, Kharitonov and Rebristy⁶, Le Borgne *et al.*⁷, Sen *et al.*⁸ and Joshi *et al.*⁹, where they have made measurements for comets P/Halley, Hartley-Good, and Austin in molecular bands, OH (3090 Å), CN (3871 Å), C_2 (5140 Å), etc. In most of the cases earlier to 1987 it is not clear whether continuum light was subtracted correctly from the measurements. In case of Comet P/Halley's recent apparition, IHW group provided similar IAU/IHW filter set to several groups and therefore it was possible to generate homogeneous data base for this comet. Subsequently, we made use of the same filter set in case of Comet Austin and now on Comet Hyakutake C/1996 B2, making it possible to compare the results on different comets.

Here we report the linear polarization measurements in some molecular bands in Comet Hyakutake C/1996 B2.

Observations and analysis

Observations were made with a photopolarimeter on the 1.2 m telescope at Gurushikhar (longitude = $72^{\circ}46'47.47''\text{E}$; latitude = $24^{\circ}39'8.84''\text{N}$; height = 1680 m above sea level) near Mount Abu, operated by Physical Research Laboratory, Ahmedabad. The PRL photopolarimeter fitted with IAU/IHW filter set was mounted at the Cassegrain plane. The polarimeter works on the principle of rapid modulation of incoming light by a fast rotating superachromatic (in $0.3\ \mu\text{m}$ to $1\ \mu\text{m}$ spectral region) Pancharatnam half wave plate¹⁰. The details of the polarimeter used are given elsewhere¹¹. The comet observations were made, with an aperture of 26.5 arcsec centered on the nucleus, on March 13.97 and March 18.88 UT decimal dates. The IAU/IHW filter set, used for observations, contains three continuum bands, 3650/80, 4845/65 and 6840/90 free from any cometary emission, and five molecular emission bands, CN (3871/50), C_3 (4060/70), CO^+ (4260/65), C_2 (5140/90) and H_2O^+ (7000/175) (all figures are in Angstrom, central wavelength/band pass). The solar type stars HD105590 and HD191854 were observed for photometric calibration. The entire photometric procedure including the measurement of flux values in different emission bands is described by Ganesh *et al.*¹² (Paper I). The details of the polarimetric analysis procedure are discussed by Joshi *et al.*¹³ (Paper II) who report the continuum polarization behaviour of the comet on the two dates.

In the emission bands, the observed flux is equal to the sum of the flux due to reflected solar continuum (F_C) and the flux due to the fluorescence emission from gas molecule (F_E). The contribution of emission polarization to the observed polarization can be found out with the help of Stokes parameters: $Q = FP \cos 2\theta$ and $U = FP \sin 2\theta$, where P is the degree of polarization, θ is position angle and F is the flux. The observed values of polarization P_{obs} and position angle θ_{obs} in emission bands are due to the mixing of emission light with continuum. Using Stokes parameters Q and U , one can estimate the emission polarization P_E and θ_E . We can write: $Q_{\text{obs}} = Q_E + Q_C$ and $U_{\text{obs}} = U_E + U_C$, where the subscript E and C stand for emission and continuum respectively. We write the final equations in the form,

$$P_{\text{obs}}F_{\text{obs}} \cos 2\theta_{\text{obs}} = P_E F_E \cos 2\theta_E + P_C F_C \cos 2\theta_C, \quad (1)$$

$$P_{\text{obs}}F_{\text{obs}} \sin 2\theta_{\text{obs}} = P_E F_E \sin 2\theta_E + P_C F_C \sin 2\theta_C, \quad (2)$$

where F_C and P_C are respectively the contribution of continuum flux in the emission band and the polarization due to this continuum.

The observed degrees of polarization in continuum and emission bands are listed in Table 1 and are plotted in Figure 1. The polarization due to continuum, P_C , in a particular emission band is estimated from Figure 1 by interpolation. The molecular band polarization P_E has been calculated using equations (1) and (2) and the values obtained are given in Table 2 along with various physical quantities needed in the calculation. The values of F_E and F_C are taken from Paper I. The value of F_{obs} is simply the sum of F_C and F_E . After inserting the known values of F_{obs} , F_C , F_E , P_{obs} , and P_C in equations (1) and (2), we get the values of $Q_E = P_E \cos 2\theta_E$ and $U_E = P_E \sin 2\theta_E$. The value of P_E is calculated from Q_E and U_E using the relation $P_E = \sqrt{(Q^2 + U^2)}$. The emission band polarization values thus obtained are listed in Table 2 and shown in Figure 2 in the form of bar diagram.

Results and discussion

The observed continuum polarization is very close to the theoretically-calculated polarization value taking grain characteristics similar to comet Halley (Paper II). In general, the polarization vector is expected to be normal to the scattering plane. However, the observed values (see Table 1) show a small deviation in the position angle in U-continuum and in CN band on March 13.97. The position angle in 3650 Å band on March 13.97 is 16 ± 2 degrees whereas in 4845 Å continuum band its value is 13 ± 1 degrees. Within the errors both the values can be taken as same. However, on March 13.97, θ deviates by about 4 degrees in 3650 Å band compared to the value in 4845 Å band, which is more than three sigma and therefore can be taken as real. This small deviation may be attributed to several factors, some of which are described in Paper II.

The polarization vector for the molecular bands is perpendicular to the scattering plane within the error of observation (c.f. Table 1). The position angle in the CN band shows a significant deviation from the normal to the scattering plane on March 13.97. However, the observed molecular band polarization is contaminated by the continuum. It would be more interesting to study the polarization behaviour of pure molecular emission which is listed in Table 2. Before we discuss the polarization behaviour of molecular bands, it is appropriate to look for the error in estimating P_E .

If we assume that emission and continuum bands have the polarization vector in the same direction (i.e. perpendicular to the scattering plane), then,

$$P_E F_E = P_{\text{obs}}(F_C + F_E) - P_C F_C. \quad (3)$$

From the observed data we notice that the above assumption is quite reasonable. We also notice that the errors in polarization measurements are extremely low (S/N ratio being more than 10); the worst case being the

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Table 1. Observed magnitudes and polarization data on Comet Hyakutake C/1996 B2 on two different dates. %P, E_p , θ , E_θ are respectively the observed degree of polarization (in %), percent error in polarization, position angle, error in position angle (degrees)

Waveband (Å)	UT decimal date March 13.97					UT decimal date March 18.88				
	%P _{obs}	E_p	θ	E_θ	Mag.	%P _{obs}	E_p	θ	E_θ	Mag.
3650*	6.38	0.50	16	2	10.30	4.98	0.31	4	2	9.65
3871 (CN)	3.65	0.49	21	3	9.24	4.58	0.31	8	2	8.69
4060 (C ₃)	4.71	0.26	10	1	9.53	4.25	0.19	6	1	8.80
4260 (CO ⁺)	5.69	0.26	11	1	9.74	5.08	0.14	7	1	8.79
4845*	5.77	0.21	13	1	8.93	5.32	0.17	8	1	8.04
5140 (C ₂)	4.84	0.19	12	1	8.49	4.52	0.13	7	1	7.86
6840*	6.32	0.29	14	1	7.90	6.26	0.24	7	1	7.46
7000 (H ₂ O ⁺)	6.47	0.25	14	1	7.82	5.74	0.15	5	1	7.04

*Continuum bands.

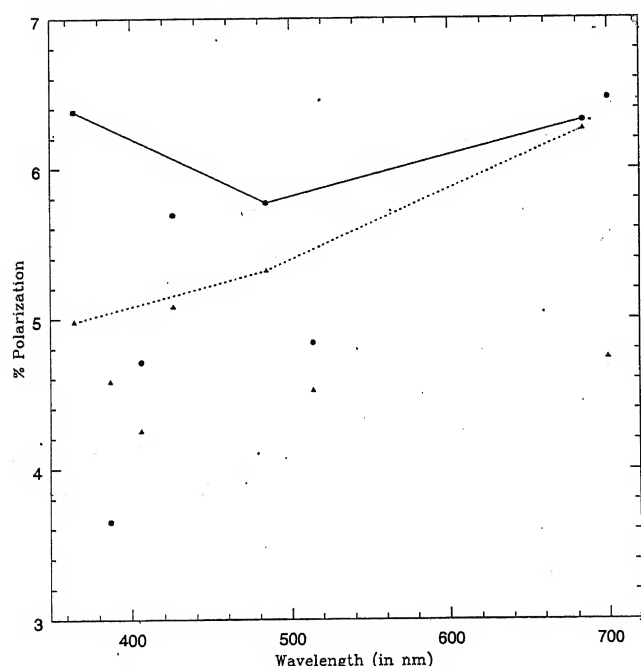


Figure 1. The observed continuum and molecular emission band polarizations plotted as a function of wavelength. The continuum points for March 13.97 and March 18.88 observations are joined by solid line and dotted line, respectively. The observed data are shown by filled circles and filled triangles for March 13.97 and 18.88 UT dates.

3650 Å band in which ϵ_p is ~0.5% (Table 1). The main source of error in the estimation of P_E is the error in flux measurements. The accuracy of flux measurement is estimated at a level of ~10%. Neglecting the error in polarization values, the expected error in molecular emission polarization can be written as

$$\delta P_E^2 = \left(\frac{\delta P_E}{\delta F_C} \right)^2 \delta F_C^2 + \left(\frac{\delta P_E}{\delta F_E} \right)^2 \delta F_E^2. \quad (4)$$

The ratio $r = F_C/F_E$ plays an important role in error estimation. If $r \gg 1$ (which is the case with CO⁺ and H₂O⁺) we can simply write,

Table 2. Values of the polarization for considered molecules in different wavebands

Waveband	March 13.97				
	M_C	F_C	F_E	P_C	P_E
3871 (CN)	10.5	9.23E-12	2.14E-11	6.38	2.48
4060 (C ₃)	9.92	1.43E-11	6.02E-12	6.17	2.02
4260 (CO ⁺)	9.77	7.18E-11	1.58E-12	6.06	48.3
5148 (C ₂)	8.88	1.95E-10	7.62E-11	5.79	2.47
7000 (H ₂ O ⁺)	7.86	2.56E-10	9.58E-12	6.41	20.04

Waveband	March 18.88				
	M_C	F_C	F_E	P_C	P_E
3871 (CN)	9.84	1.75E-11	3.32E-11	5.03	4.41
4060 (C ₃)	9.19	2.79E-11	1.18E-11	5.11	2.40
4260 (CO ⁺)	8.99	1.46E-10	3.04E-11	5.15	5.52
5140 (C ₂)	8.02	4.19E-10	6.89E-11	5.37	1.30
7000 (H ₂ O ⁺)	7.45	3.75E-10	1.71E-10	6.06	5.49

$$\delta P_E \sim r ((P_{\text{obs}} - P_C)/F_E) \delta F_E. \quad (5)$$

Using the values from Table 2 we see that r is rather large for the ionic molecules CO⁺ (~50) and H₂O⁺ (~30) on March 13.97, and therefore the emission band polarization for ions derived by this method has large errors. Therefore, the values of P_E and θ_E for CO⁺ and H₂O⁺ molecules on March 13.97, as listed in Table 2, may contain large errors and should be taken with caution. The CO⁺ shows very high polarization value (48%). In case of Comet Halley also, CO⁺ showed large polarization⁸, though in that case also the errors were large. However, for other molecules the value of r is about 1 and therefore δP_E is small.

The value of P_E for CN, C₂ and C₃ molecules is estimated to be less than 3% on March 13.97. However, on March 18.88 polarization values for CN, C₃ and C₂ molecules are 4.41, 2.40 and 1.30, respectively. Thus neutral CN molecule shows higher polarization (4.58%) than one would expect from theory as discussed later but the value of P_E for C₂ band comes out to be lower than expected. This could be due to the overestimation of continuum polarization flux. Since the error in the de-

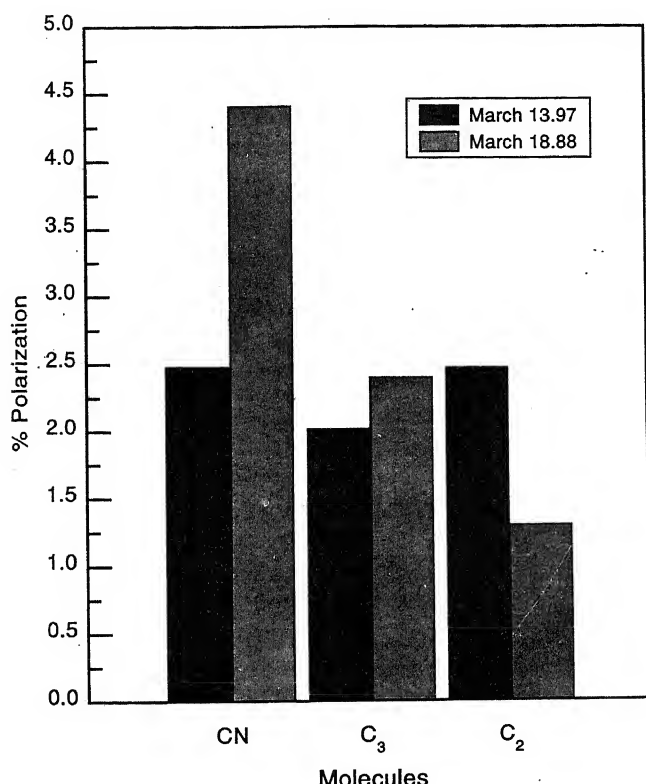


Figure 2. Bar chart showing a comparison of emission band polarization for CN, C₃ and C₂ molecules on March 13.97 and March 18.88.

gree of polarization is very small ($\approx 0.24\%$), the major source of error seems to be overestimation of the continuum flux at 5140 \AA obtained using formulae given in IHW Circular 3 Feb. 1984 which are basically for solar type flux distributions. The energy distribution of the comet on March 18.88 is found to be reddened (Paper I) and also peaking near 4845 \AA compared to the solar analog. In the interpolation formulae to estimate continuum flux at 5140 \AA , more weight is given to continuum flux at 4845 \AA . Therefore, the flux gets overestimated in this procedure and the angle is also found to be significantly deviated from the normal to scattering plane. On the other hand, we estimated flux by linear interpolation between 4845 and 6850 \AA , which comes out to be $\approx 2.559 \times 10^{-10}$ in the 5140 \AA band. Using this value of flux, we obtain polarization value as 4.5% and angle θ as 7° , which are closer to theoretical predictions. The actual value for the molecular polarization is expected to lie between 1.5 and 4.5% .

The molecular band polarization $P(\alpha)$ observed at a particular sun-comet-earth phase angle α , is supposed to follow the following theoretical relation³,

$$P(\alpha) = \frac{P_{\max} \sin^2 \alpha}{(1 + P_{\max} \cos^2 \alpha)}, \quad (6)$$

where P_{\max} is the maximum polarization observed at a phase angle 90° . At the time of our observations, the phase angles were 36.9° and 38.1° respectively on March 18 and March 13. Theoretical calculations carried out by Ohman³ predicted a value of 7.7% for P_{\max} for CN and C₂ molecules. Putting this value of P_{\max} in the above relation, we get $P_\alpha \approx 2.7\%$ and 2.8% respectively for phase angles 36.9 and 38.1° for CN and C₂ molecules. Therefore, the observed values are in good agreement with the theoretically calculated values. The observed molecular band polarization values for C₃ are 2.02 and 2.40% on March 13.97 and 18.88, respectively which are less than theoretically predicted value of $\sim 6\%$ (P_{\max} taken as 19%). Similar values of polarization for C₃ band have been reported for Comet Halley and Comet Austin⁷⁻⁹.

Conclusions

In the present work we have reported molecular emission band polarization in Comet Hyakutake C/1996 B2. The observations were taken on two UT dates: March 13.97 and March 18.88 using IAU/IHW set of filters. The errors in the measurement of the percent polarization are very low ($<0.5\%$). The values of the polarization obtained in the present work and those predicted theoretically are in fairly good agreement for CN and C₂ molecules. However, in case of C₃ molecule the measured polarization values do not agree with the theoretically predicted value of $P_{\max} = 19\%$.

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Organochlorine pesticides and preterm labour in human beings

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In this study, we compare organochlorine pesticides, viz. aldrin, isomers of HCH, metabolites of DDT and heptachlor in the circulating blood, cord blood and placenta of pregnant women undergoing full term normal delivery with those in premature labour. The data and the statistical outcome of the study show that the mean concentrations of organochlorine pesticides in maternal blood of cases of premature labour were not found to be significantly different from those of controls (full term normal delivery). Similar results were obtained from the residue levels of cord blood and placental tissue. An attempt has also been made to correlate the high concentration of chlorinated hydrocarbon pesticides with the initiation of premature labour on the basis of existing evidences.

THE widespread use of organochlorine compounds as insecticides during the past few decades has led to their ubiquitous presence in the environment. These compounds are highly lipid soluble and are resistant to environmental degradation.

In human beings these compounds are stored in fat-rich tissues and are resistant to metabolism. These substances are present in women and the foetus is exposed during *in utero* development by transplacental transfer. Exposure of the foetus is related to the maternal body

burden. Several studies have reported values of organochlorine pesticides in maternal blood and cord blood¹⁻⁸. Relatively high residue levels of organochlorine pesticides have been reported in women with premature delivery⁹⁻¹¹. Also DDT residue levels were higher in California sea lions which gave birth prematurely than in those with full term pups¹². Organochlorine pesticides may disturb the hormonal balance of pregnancy and perhaps precipitate labour. Some DDT analogs (p-p'-DDT and o-p'-DDT) are reported to have oestrogenic effect^{13,14}. It is also reported that oestrogen and progesterone metabolism is greatly enhanced by the hepatic microsomal xenobiotic metabolizing enzymes stimulated by chlorinated hydrocarbons¹¹. Thus, an elevated level could theoretically cause premature labour by decreasing the amount of available progesterone which maintains the homeostasis of pregnancy. The report further states that DDT and p-p'-DDE were found in all cases of abortion but no correlation was found between the accumulation of organochlorine pesticides and incidence of labour¹¹. In the present study aldrin, HCH and its isomers, metabolites of DDT and heptachlor in the maternal blood, cord blood and placenta of pregnant women undergoing full term normal delivery are compared with those in premature labour.

The women subjects, 19 to 34 years of age admitted into Mahila Chikitsalya, attached to Department of Obstetrics and Gynaecology, SMS Medical College, Jaipur were used in the present study. Eight women went into labour during 24-32 weeks of gestation and were considered as cases of premature birth. The remaining 22 women had normal full term labour and were used as controls. In general they had no history of any occupational or accidental exposure to pesticides. However, they were asked to fill up a questionnaire incorporating

Table 1. Levels of organochlorine pesticides (ppb) (mean, S. E. and range) in maternal blood of women undergoing full term labour and women who had premature labour

Organochlorine pesticide compounds	FTND (n = 22)			PL (n = 8)			Statistical significance
	Mean \pm S.E.	Range and no. of positive samples		Mean \pm S.E.	Range and no. of positive samples		
α -HCH	124.7 \pm 19.31	(14.0–280.0)	(n = 20)	107.1 \pm 39.49	(18.0–286.0)	(n = 7)	NS
γ -HCH	62.4 \pm 23.94	(9.0–491.0)	(n = 20)	22.0 \pm 5.43	(14.0–38.0)	(n = 4)	NS
β -HCH	126.6 \pm 60.11	(18.0–954.0)	(n = 15)	96.2 \pm 21.28	(47.0–151.0)	(n = 4)	NS
Heptachlor	1582.8 \pm 315.36	(103.0–2563.40)	(n = 15)	1031.8 \pm 320.99	(154.0–2555.0)	(n = 7)	NS
Aldrin	182.4 \pm 26.35	(2.0–352.0)	(n = 20)	95.8 \pm 46.67	(4.6–351.0)	(n = 7)	NS
Heptachlor epoxide	860.2 \pm 224.97	(46.0–3653.0)	(n = 22)	564.6 \pm 452.55	(46.0–2362.0)	(n = 5)	NS
p-p'-DDE	83.5 \pm 14.60	(7.0–222.0)	(n = 21)	81.8 \pm 26.88	(11.0–194.0)	(n = 7)	NS
p-p'-DDD	16.9 \pm 4.69	(0.001–47.0)	(n = 12)	30.5 \pm 15.54	(15.0–46.0)	(n = 2)	NS
p-p'-DDT	35.0 \pm 8.68	(1.0–112.0)	(n = 16)	32.1 \pm 18.667	(1.0–102.0)	(n = 6)	NS
Σ HCH	245.6 \pm 56.12	(69.0–1077.0)	(n = 22)	125.5 \pm 33.39	(30.0–312.0)	(n = 8)	NS
Σ Heptachlor	1911.9 \pm 425.71	(46.0–8875.0)	(n = 22)	842.7 \pm 304.64	(45.0–2825.0)	(n = 8)	NS
Σ DDT	120.2 \pm 19.03	(10.0–330.0)	(n = 21)	88.5 \pm 15.87	(27.0–145.0)	(n = 8)	NS
Σ OCP	2420.2 \pm 465.73	(10.0–9698.0)	(n = 22)	1083.0 \pm 310.18	(332.0–3108.0)	(n = 8)	NS

FTND, Full term normal delivery; PL, preterm labour; Σ HCH, Total HCH equivalent; Σ Heptachlor, Total Heptachlor equivalent; Σ DDT, Total DDT equivalent; Σ OCP, Total organochlorine pesticides equivalent; NS, Not significant.

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Table 2. Levels of organochlorine pesticides (ppb) (mean, S. E. and range) in cord blood of women undergoing full term labour and women who had premature labour

Organochlorine pesticide compounds	FTND (<i>n</i> = 22)			PL (<i>n</i> = 8)			Statistical significance
	Mean ± S.E.	Range and no. of positive samples		Mean ± S.E.	Range and no. of positive samples		
α-HCH	150.8 ± 19.39	(5.0–508.0)	(<i>n</i> = 21)	189.2 ± 50.92	(52.0–454.0)	(<i>n</i> = 7)	NS
γ-HCH	38.6 ± 7.57	(1.0–95.0)	(<i>n</i> = 27)	40.0 ± 5.78	(22.0–56.0)	(<i>n</i> = 5)	NS
β-HCH	167.2 ± 119.02	(2.0–1945.0)	(<i>n</i> = 16)	98.1 ± 23.57	(20.0–187.0)	(<i>n</i> = 6)	NS
Heptachlor	1420.9 ± 193.62	(90.0–2586.0)	(<i>n</i> = 15)	1328.1 ± 677.47	(176.0–5484.0)	(<i>n</i> = 7)	NS
Aldrin	129.2 ± 35.44	(3.0–481.0)	(<i>n</i> = 21)	95.7 ± 28.04	(6.0–236.0)	(<i>n</i> = 7)	NS
Heptachlor epoxide	864.8 ± 219.14	(15–3169.0)	(<i>n</i> = 16)	650.1 ± 158.42	(201.0–1188.0)	(<i>n</i> = 6)	NS
p-p'-DDE	64.6 ± 15.11	(6.0–211.0)	(<i>n</i> = 20)	81.3 ± 18.77	(16.0–195.0)	(<i>n</i> = 8)	NS
p-p'-DDD	57.6 ± 48.83	(0.001–300.0)	(<i>n</i> = 6)	23.3 ± 6.94	(10.0–34.0)	(<i>n</i> = 3)	NS
p-p'-DDT	44.8 ± 22.00	(0.001–288.0)	(<i>n</i> = 16)	8.2 ± 2.86	(0.001–17.07)	(<i>n</i> = 5)	NS
Σ HCH	305.5 ± 112.54	(7.0–2453.0)	(<i>n</i> = 21)	264.2 ± 71.65	(52.0–697.0)	(<i>n</i> = 8)	NS
Σ Heptachlor	1734.4 ± 27.09	(15.0–4001.0)	(<i>n</i> = 20)	1749.8 ± 737.34	(176.0–6327.0)	(<i>n</i> = 8)	NS
Σ DDT	98.8 ± 34.53	(0.001–721.0)	(<i>n</i> = 21)	94.8 ± 21.93	(33.0–229.0)	(<i>n</i> = 8)	NS
Σ OCP	2101.3 ± 367.21	(15.0–6317.0)	(<i>n</i> = 22)	2175.8 ± 772.39	(4.0–6934.0)	(<i>n</i> = 8)	NS

FTND, Full term normal delivery; PL, preterm labour; Σ HCH, Total HCH equivalent; Σ Heptachlor, Total Heptachlor equivalent; Σ DDT, Total DDT equivalent; Σ OCP, Total organochlorine pesticides equivalent; NS, Not significant.

Table 3. Levels of organochlorine pesticides (ppb) (mean, S. E. and range) in placenta of women undergoing full term labour and women who had premature labour

Organochlorine pesticide compound	FTND (n = 22)			PL (n = 8)			Statistical significance
	Mean \pm S.E.	Range and no. of positive samples		Mean \pm S.E.	Range and no. of positive samples		
α -HCH	107.0 \pm 26.0	(9.0–369.0)	(n = 21)	112.0 \pm 22.45	(68.0–210.0)	(n = 6)	NS
γ -HCH	22.6 \pm 4.87	(1.0–76.0)	(n = 15)	39.2 \pm 14.37	(3.0–107.0)	(n = 8)	NS
β -HCH	202.7 \pm 141.16	(15.0–1879.0)	(n = 13)	71.7 \pm 21.88	(13.0–171.0)	(n = 8)	NS
Heptachlor	1297.5 \pm 242.15	(232.0–2808.0)	(n = 14)	835.2 \pm 250.00	(406.0–1876.0)	(n = 4)	NS
Aldrin	247.3 \pm 35.45	(5.0–494.0)	(n = 18)	364.0 \pm 12.14	(20.0–84.0)	(n = 5)	NS
Heptachlor epoxide	917.6 \pm 225.9	(15.0–2789.0)	(n = 15)	307.7 \pm 153.34	(9.0–688.0)	(n = 4)	NS
p-p'-DDE	276.3 \pm 185.45	(1.0–3766.0)	(n = 20)	40.2 \pm 8.58	(11.0–75.0)	(n = 8)	NS
p-p'-DDD	22.6 \pm 13.23	(2.0–73.0)	(n = 5)	132.5 \pm 123.86	(9.0–256.0)	(n = 2)	NS
p-p'-DDT	93.9 \pm 31.70	(4.0–415.0)	(n = 13)	13.2 \pm 5.35	(0.001–29.0)	(n = 5)	NS
Σ HCH	237.1 \pm 89.11	(11.0–981.0)	(n = 22)	195.7 \pm 50.49	(16.0–488.0)	(n = 8)	NS
Σ Heptachlor	1595.2 \pm 296.40	(15.0–5024.0)	(n = 20)	914.4 \pm 309.18	(9.0–2293.0)	(n = 5)	NS
Σ DDT	321.9 \pm 195.64	(1.0–4181.0)	(n = 21)	82.0 \pm 32.68	(19.0–298.0)	(n = 8)	NS
Σ OCP	2195.5 \pm 389.42	(25.0–5734.0)	(n = 22)	868.3 \pm 288.76	(184.0–2635.0)	(n = 8)	NS

FTND, Full term normal delivery; PL, preterm labour; Σ HCH, Total HCH equivalent; Σ Heptachlor, Total Heptachlor equivalent; Σ DDT, Total DDT equivalent; Σ OCP, Total organochlorine pesticides equivalent; NS, Not significant.

factors relevant to pesticide residue accumulation such as social status, dietary habits, area of residence, weight, parity, age, habits of pesticides use, etc.

Five ml of maternal blood from each case was collected by venipuncture in preheparinized vials 4–8 hours before parturition. A fraction of placental tissue was collected in acetone-washed aluminium foil at the time of delivery. Umbilical cord blood was collected by squeezing the cord into preheparinized vials. All the samples were stored at -10°C in deep freeze and analysed within 48 hours of their storage.

Pesticides were extracted and separated from samples by liquid partition and column chromatography so they may be analysed by GLC and TLC procedures.

Pesticides from maternal blood/cord blood extracted by a method given by Bush and his coworkers in 1984 with little modifications¹⁵. To 2 ml of maternal blood or neonatal blood, methanol (5 ml) and 1:1 diethyl ether/hexane (8 ml) were added and the contents were shaken for 3 minutes by hand. Loss of solvent due to evaporation was assessed by weighing the tube before and after shaking. The contents were then centrifuged for 10 minutes at 3000 rpm. It was re-extracted by adding 8 ml 1:1 diethyl ether/hexane. Pooled solvent so obtained contains the lipophilic organochlorine pesticides.

The method adopted for the extraction of pesticides from the placental tissue is nearly the same as that described for the blood since the main contents of placenta

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are blood and blood capillaries. Two g of placental tissue was homogenized in pestle and mortar with a little sea sand¹⁵. All reagents and chemicals used were of HPLC and analytical grade and checked for any pesticide contamination.

Florosil column was used for the clean up of extracted samples. It was prepared essentially in the same way as described by Bush and his coworkers¹⁵.

A Hewlett Packard 5890 series II gas chromatograph equipped with ⁶³N foil electron capture detector (ECD) and coupled with an integrator, HP 3396 A was used for the analysis of samples. Purified nitrogen (IOLAR-I) was used as the carrier gas. A coiled glass column (1.43 m × 4 mm L × I.D.) was packed with solid support, chromosorb 100/120 mesh size along with the liquid phase 1.5% OV-17 + 1.95% OV-210. A known volume of the sample was injected into the column with the help of a 10 µl Hamilton syringe. The different peaks in the samples were identified by comparing their retention times with those of the standards. Quantitation of the samples was done by the data obtained from the integrator and were based on peak areas. Standards were obtained from Environmental Protection Agency (EPA), USA.

Detected pesticides were further confirmed by thin layer chromatography. Since, sensitivity range of TLC is much less than GLC, therefore extracted and cleaned samples after their analysis on GLC were concentrated prior to their use in TLC.

The calculations are based on biological statistics and values are expressed as mean ± standard error (S.E.). The difference in the pesticide residue level between different groups was analysed with the help of Student's *t* test. Significance between the residue levels of different groups was judged at 5% and 1% levels.

The concentration of organochlorine pesticides was estimated in the maternal blood, cord blood and placental tissue of each subject undergoing premature labour and normal delivery and the data and statistical outcome of the study are shown in Tables 1–3. The mean concentration of organochlorine pesticides in maternal blood of cases of premature labour was not found to be significantly different from that of controls (full term normal delivery). Similar results were obtained from the residue levels of cord blood and placental tissue.

The data and the statistical outcome of the study show that mean concentrations of organochlorine pesticides in maternal blood, cord blood and placental tissue of premature labour were not found to be significantly different from those of controls (full term normal delivery). Our findings are contradictory to the findings of O'Leary and his coworkers¹¹ from Florida in which mean and range of DDE in foetal whole blood from healthy white controls were 4.9 ppb (2 to 13 ppb) while the values in the premature group were 22.1 ppb (18.7 to 26.8 ppb). Similar findings were observed in the

Negro infants. The Negro premature group had a mean of 6.1 ppb and range of 3 to 12 ppb, while the Negro premature infant had a mean of 19.0 ppb and range 6.6 to 34.4 ppb. The above results suggested the involvement of high levels of DDE in the initiation of premature labour.

Our results do not coincide with the findings of Siddique¹⁶ in which he reported that relatively high levels of organochlorine pesticides, especially DDT and DDE in cases of premature labour as compared to full term normal labour in the specimen of maternal blood and placental tissue, could suggest the involvement of these pesticides in the termination of pregnancy.

Since, we could not find any differences in the organochlorine pesticide accumulation between preterm and full term cases, our findings suggest that there are some factors other than pesticides such as stress, high blood pressure, poor nutrition, excessive physical activity, smoking, infections, teenage pregnancy, anaemia and other medical conditions such as diabetic nephropathy and uterine anomalies which are responsible for the initiation of preterm labour.

Data is not stratified with age, parity, dietary habits, weight, social status, area of residence and pesticide use habits because there were only limited number of cases of premature labour.

Hence, from the present study it is not clear whether the organochlorine pesticides play any role in the initiation of preterm labour (Tables 1–3). Therefore, further studies are to be conducted in order to draw any conclusion about the antagonistic role of organochlorine pesticides in pregnancy.

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Transfer factors of radionuclides ^{137}Cs and ^{65}Zn from soil to pearl millet and sorghum

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The soil to plant transfer factors (TF) of ^{137}Cs and ^{65}Zn were determined for two crops, sorghum and pearl millet, under irrigated conditions in greenhouse and under rainfed conditions in field. In the greenhouse experiment, the accumulation of ^{137}Cs was almost doubled when the soil contamination level was doubled. Under field conditions, ^{137}Cs concentration in both pearl millet and sorghum grains as well as straw was nearly four times more at 148 kBq kg⁻¹ level of soil contamination as compared to lower level of 74 kBq kg⁻¹ soil. The TF values for ^{65}Zn determined under greenhouse conditions for both the crops were nearly a hundred-fold higher as compared to ^{137}Cs .

AFTER the Chernobyl nuclear station accident in 1986, worldwide concern has grown to evaluate the dose of radiation which may affect man. The *Handbook of Parameter Values* for prediction of radionuclide transfer in the ecosystems published by the International Atomic Energy Agency¹ is based on data entirely for crops, animals and conditions found in temperate climate zones. Very few data are available for radionuclide transfer and uptake in tropical and subtropical ecosystems. In the published literature, data for the uptake of radionuclides from soil for tropical cereals, fruits, herbs, tea and root crops are few in numbers and primarily limited to Cs. Although more data are available for rice, due to the complicated nature of production and the number of varieties, the data are not consistent. Currently it is assumed that transfers to animal and man are similar to those in temperate environments. Whilst this

assumption is probably not unreasonable, it has never been validated.

Keeping these points in view, a study has been initiated to assess the transfer of ^{137}Cs and ^{65}Zn from soil artificially contaminated with these radionuclides to pearl millet and sorghum under rainfed conditions in field and under irrigated conditions in pot culture.

The field experiment was conducted in the Gamma Garden of the Indian Agricultural Research Institute research farm in 1994 kharif season. In field experiments in the main yield plots of 9 m² (3 m × 3 m), microplots of 1 m² (1 m × 1 m) were made and contaminated with 74 kBq kg⁻¹ soil (2 µCi kg⁻¹ soil) and 148 kBq kg⁻¹ soil (4 µCi kg⁻¹ soil) with ^{137}Cs . The entire upper 5 cm soil of the microplots (75-80 kg) was dug out and sprayed with the 500 ml solution of radionuclide containing the required amount of activity. The radionuclides were mixed thoroughly with the entire soil and the soil was then transferred back to the microplots and brought to 50% water saturation level to a depth of 20 cm. Radionuclides were allowed to equilibrate in soil for eight weeks and during this period the soil was kept between 35 and 50 per cent moisture saturation.

After equilibration period, pearl millet variety M-179 and sorghum variety PC-121 were grown with a row-to-row spacing of 40 cm and plant-to-plant spacing of 20 cm. The crops were fertilized with 80 kg N ha⁻¹ through urea applied in two splits, 40 kg P₂O₅ ha⁻¹ through single superphosphate and 40 kg K₂O ha⁻¹ through muriate of potash applied as basal.

As maturity, the plants were harvested and separated into grain and straw. In grain and straw samples from microplots, ^{137}Cs activity was measured using a 3" × 3" NaI (T1) flat type detector for ^{137}Cs , 0.661 MeV peak as per the procedures given in the IAEA Technical Report². The soil samples were also drawn from microplots to a depth of 20 cm. Though the upper 5 cm soil was contaminated with radionuclide, the soil samples were collected to a depth of 20 cm from six locations in each microplots with a Viehmeyer tube and pooled to a composite sample as per the procedure described in IAEA Technical Report². The soil samples were air dried, ground in a wooden pestle mortar and counted in a well type NaI (T1) detector (2.5" × 2.5"). The counting efficiency for ^{137}Cs was 0.881% for flat type detector and 11.82% for the well type detector.

A similar experiment was conducted under pot culture conditions in the same soil. Eight kg soil was taken in ceramic pots and contaminated with ^{137}Cs and ^{65}Zn at the rate of 148 kBq kg⁻¹ soil and 296 kBq kg⁻¹ soil, respectively. In equilibrated soils, four seeds of pearl millet or sorghum were sown and on germination the plants were thinned to two in each pot. Here the results on transfer factors of ^{137}Cs under both field and pot culture conditions and of ^{65}Zn in pot culture condition are presented. The data on soil to plant transfer factors of the

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radionuclides were computed as ratios of (Bq kg⁻¹ dry-matter or grain)/(Bq kg⁻¹ dry soil).

In the greenhouse experiment, the pearl millet and sorghum plants were harvested at maturity and separated into grain and straw. The grain and straw yields of pearl millet were 9.34 ± 1.75 and 22.46 ± 2.33 g/pot, respectively. The similar figures for sorghum were 11.27 ± 1.38 and 27.62 ± 3.45 g/pot, respectively. The data for ¹³⁷Cs transfer factors presented in Table 1, revealed that under greenhouse conditions at lower level of soil contamination with ¹³⁷Cs (148 kBq kg⁻¹ soil) it

Table 1. Transfer factor of ¹³⁷Cs in pearl millet and sorghum grain and straw under pot and field conditions

Soil contamination level (kBq kg ⁻¹)		Plant contamination (Bq kg ⁻¹)	Transfer factor (×10 ³)
Pearl millet (Pot experiment)			
145.8 ± 1.43	Grain	877 ± 188	6.01 ± 1.24
	Straw	1723 ± 331	11.80 ± 2.17
293.6 ± 1.20	Grain	1318 ± 133	4.49 ± 0.47
	Straw	1874 ± 60	6.38 ± 0.22
Sorghum (Pot experiment)			
146.3 ± 0.33	Grain	662 ± 23	4.52 ± 0.15
	Straw	1832 ± 56	12.52 ± 0.40
295.3 ± 0.43	Grain	1714 ± 580	5.80 ± 1.96
	Straw	2651 ± 659	8.98 ± 2.22
Pearl millet (Field experiment)			
18.48 ± 0.09	Grain	39.7 ± 8.7	2.15 ± 0.48
	Straw	61.0 ± 9.6	3.31 ± 0.49
36.74 ± 0.10	Grain	163.3 ± 5.1	4.45 ± 0.13
	Straw	312.3 ± 33.3	8.49 ± 0.90
Sorghum (Field experiment)			
18.31 ± 0.07	Grain	36.7 ± 14.7	2.01 ± 0.81
	Straw	74.7 ± 12.1	4.09 ± 0.67
36.84 ± 0.22	Grain	226.3 ± 11.4	6.13 ± 0.28
	Straw	255.0 ± 11.1	6.91 ± 0.27

Table 2. Transfer factor of ⁶⁵Zn in pearl millet and sorghum grain and straw under pot culture conditions

Soil contamination level (kBq kg ⁻¹)		Plant contamination (kBq kg ⁻¹)	Transfer factor (×10 ¹)
Pearl millet			
145.5 ± 2.98	Grain	101.0 ± 22.3	6.96 ± 1.63
	Straw	56.8 ± 8.5	3.91 ± 0.61
293.6 ± 1.21	Grain	95.2 ± 15.3	3.24 ± 0.52
	Straw	87.3 ± 9.0	2.97 ± 0.30
Sorghum			
146.3 ± 0.33	Grain	63.5 ± 0.7	4.34 ± 0.05
	Straw	82.8 ± 2.2	5.66 ± 0.16
295.3 ± 0.44	Grain	117.7 ± 1.3	3.98 ± 0.03
	Straw	150.5 ± 10.3	5.10 ± 0.35

was 6.01×10^{-3} and 11.80×10^{-3} in pearl millet grain and straw respectively. At higher level of soil contamination (296 kBq kg⁻¹ soil), the transfer factors were much less both in grain and straw, the values being 4.49×10^{-3} and 6.38×10^{-3} , respectively. The transfer factor values for sorghum grain were 4.52×10^{-3} and 5.80×10^{-3} respectively, and for sorghum straw, 12.52×10^{-3} and 8.98×10^{-3} respectively at single or double levels of soil contamination with ¹³⁷Cs. Gerzabek *et al.*³ have reported from two field studies at two sites contaminated by Chernobyl fallout, the transfer factor values for potato tuber of 1.7×10^{-3} and for wheat straw 0.7×10^{-2} . McCee *et al.*⁴ calculated the plant soil concentration ratio, transfer factor and plant-plant ratios from fallout ¹³⁷Cs data of soil and plants and found that the greatest spread was associated with plant soil transfer factors. In absolute terms, however, the accumulation of ¹³⁷Cs in pearl millet and sorghum grains (Bq kg⁻¹ grain) almost doubled when the soil contamination level was doubled.

In the field experiment, the grain yields of pearl millet and sorghum were 24.14 ± 0.99 , and 22.44 ± 1.08 and straw yields were 73.67 ± 3.37 and 89.42 ± 4.24 q/ha, respectively. Under field conditions the transfer of ¹³⁷Cs from soil to pearl millet grain was 2.15×10^{-3} and 4.45×10^{-3} at lower level (74 kBq kg⁻¹ soil) and higher level (148 kBq kg⁻¹ soil) of ¹³⁷Cs contamination of soil, respectively. In sorghum grain the transfer factor of ¹³⁷Cs was 2.01×10^{-3} and 6.13×10^{-3} at lower and higher levels of soil contamination. Similar trend was observed in both pearl millet and sorghum straw. However in absolute figures (Bq kg⁻¹ dry matter), the ¹³⁷Cs concentration in both pearl millet and sorghum grain as well as straw was nearly four to six times more at higher level of soil contamination (148 kBq kg⁻¹ soil) compared to that at lower level of 74 kBq kg⁻¹ soil.

The transfer factor values of ⁶⁵Zn determined under greenhouse conditions and presented in Table 2, were found nearly a hundred fold higher than compared to ¹³⁷Cs for both pearl millet and sorghum. As an example, at lower level of soil contamination with ⁶⁵Zn (148 kBq kg⁻¹ soil or 4 µCi kg⁻¹ soil), it was 6.96×10^{-1} for pearl millet grain and 4.34×10^{-1} for sorghum grain respectively. The probable reason for higher transfer factor for ⁶⁵Zn observed is that, zinc being an essential plant nutrient is absorbed by plant roots in large amount and translocated to above ground portions, while ¹³⁷Cs enters the plant system along with potassium. A similar trend is also reported earlier for rice crop by D'Souza and Mistry⁵. However, the transfer factors for ¹³⁷Cs and ⁶⁵Zn reported here are lower than for rice crop under field capacity moisture regime, probably due to the differences in soil properties of tropical laterite, black and alluvial soils. Further, in absolute amounts, the ⁶⁵Zn concentration was higher in pearl millet grain than in straw portion, whereas in sorghum it was otherwise,

more in straw than in grain. It has been reported by Tulin *et al.*⁶ from a study in soils contaminated due to Chernobyl accident that the increasing levels of potassium fertilization reduce the absorption of ¹³⁷Cs from soil by oats. Similar observations were reported by Orlovius and Sattler⁷. Although in the present investigation the crop was fertilized with 40 kg K₂O ha⁻¹ and has illite as the dominating clay mineral, the contamination level did not show a decline in soil to plant transfer of ¹³⁷Cs in both pearl millet and sorghum.

The results reported here indicate that release of considerable amounts of radionuclides from nuclear facilities resulting in contamination of soils, may find their way into crops in the tropical regions and eventually into the food chain of man.

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Stylar length variation in *Caesalpinia pulcherrima* (Caesalpinaceae) – Basic patterns

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Caesalpinia pulcherrima (Caesalpinaceae) produces four categories of morphologically distinguishable flowers within the same inflorescence, viz. flowers with long, medium, short and rudimentary styles. The stylar length variation within an inflorescence was found to be discontinuous. The long-styled flowers were functionally hermaphroditic and are produced towards the basal region of inflorescences whereas medium, short and rudimentary styled flowers were essentially males and are produced towards top region. The adaptive significance of this variation has been discussed in the light of stochastic events of resource availability and plant-pollinator interaction.

STYLE lengths of flowers borne on different individuals vary notably among species exhibiting di and tri-stylous condition^{1,2}. Among andromonoecious species, male plants bear flowers with rudimentary styles while flowers of hermaphrodite plants possess normal styles^{3–5}. However, among angiosperms, perfect flowers borne on a plant or even within an inflorescence also exhibit subtle differences in floral morphology and/or function. For instance, flowers of *Callonia grandiflora* (Polemoniaceae) exhibit intra-individual differences with respect to style length, pollen tube growth rate and

morphology of stigmatic papillae⁶. Cruden and Hermann-Parker⁷ have shown that *Caesalpinia pulcherrima* produces two types of flowers within an inflorescence: hermaphroditic flowers with abundant nectar and normal style; male flowers with rudimentary style and poor nectar.

Of late, floral variations within an inflorescence have been viewed as a result of dynamic interaction among plants and their pollinators. In a recent study Ganeshiah *et al.*⁸ have shown that figs guard their flowers against depredation from agonid wasps by varying the stylar length among flowers within a synconium. Stochastic events of fruit set and availability of resources to developing young buds within an inflorescence may also determine the extent of variation in floral features. Hence intra-inflorescence variation in floral features may be more common in flowering plants and might also represent an important mechanism to increase pollination efficiency. However, studies documenting such variations are scanty.

We have attempted to assess variation in stylar length within inflorescences in *Caesalpinia pulcherrima* (Caesalpinaceae), its association with floral functioning and also the possible adaptive significance of such variations.

The experiment was undertaken at College of Agriculture, Raichur (16°15'N, 77°20'E; 389 m above MSL), Karnataka, India.

Caesalpinia pulcherrima (Caesalpinaceae) is a perennial woody shrub of Indo-Malayan origin⁹. Plants bear either bright red or yellow flowers throughout the year. The flowers exhibit psychophilic syndrome and hence are pollinated by butterflies and moths⁷. In *Caesalpinia*, the inflorescence is a compound raceme with primary, secondary, tertiary and quaternary inflorescences emerging from a common axis and blooming occurs in

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an acropetal succession (basal floral buds bloom first and proceed in upward direction). The flowers anthesis around 8.00 am and are retained on the inflorescence for well over two days. The older flowers change their colour to deeper shades. Generally fruit set is maximum in primary inflorescences followed by secondary inflorescences. In most cases tertiary and quaternary inflorescences fail to produce fruits.

During 1993 in each of the 18 plants found growing in the campus (nine with yellow and nine with red flowers), six randomly chosen inflorescences were tagged before blooming. The flowers were observed for the stylar character (length) throughout the blooming period and grouped into four categories, viz. long (if the style length was > 4 cm, extend above or at the same height of the anthers), medium (if the style length was between 3 and 4 cm), short (style length between 1 and 3 cm) and rudimentary (< 1 cm). The observations were repeated during 1994 for the same set of plants.

To assess the function of different styled flowers, they were separately pollinated artificially. Pollen derived from them was analysed for their size and percent fertility using acetocarmine stain test². The number of ovules per ovary was counted under a stereo-binocular dissection microscope.

Means were compared between groups by Student's *t* test, the frequency distribution by Kolmogorov Smirnov Test¹⁰.

Caesalpinia pulcherrima produces four morphologically distinguishable flowers varying in stylar length within an inflorescence (Table 1). Although the style length varies from 0.4 to 6.5 cm, flowers could be categorized into four groups viz. long styled (mean \pm SE = 5.67 ± 0.046 & 5.74 ± 0.048 for yellow and red morph respectively), medium (3.79 ± 0.110 ; 3.44 ± 0.143), short (2.06 ± 0.058 ; 2.09 ± 0.042) and rudimentary (0.65 ± 0.022 ; 0.67 ± 0.020) (Table 1). The frequency distribution of stylar length was multi-moded and departed statistically from the normal distribution (Table 1 and Figure 1; KS tests were significant with $P < 0.001$ between frequency distributions). The mean stylar length of each of these categories did not differ between red and yellow morphs but differed significantly within a morph (Table 1).

Table 1. Stylar length variation (in cm) in *Caesalpinia*

Flower type	Yellow		Red	
	<i>n</i>	Mean \pm SE	<i>n</i>	Mean \pm SE
Long styled	67	$5.67^a \pm 0.046$	56	$5.74^a \pm 0.048$
Medium styled	25	$3.79^b \pm 0.110$	20	$3.44^b \pm 0.143$
Short styled	44	$2.06^c \pm 0.058$	63	$2.09^c \pm 0.042$
Rudimentary styled	40	$0.65^d \pm 0.022$	67	$0.67^d \pm 0.020$

Mean values with same superscript (within a flower morph) do not differ statistically ('*t*' test significant $P < 0.001$).

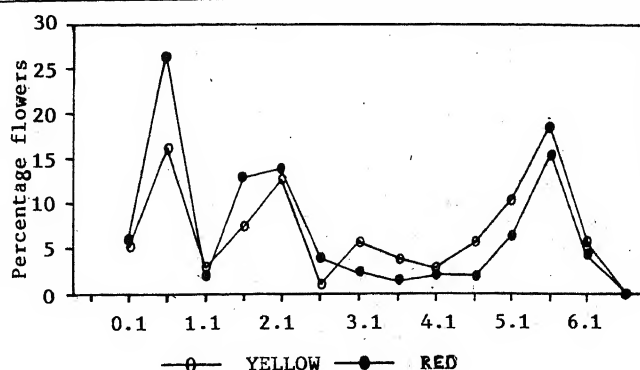


Figure 1. Distribution of stylar lengths of *Caesalpinia* (Yellow morph – open circle; red morph – closed circle).

Table 2. Result of artificial crossing/selfing between the flower groups in *Caesalpinia*

Category	Fruit set
Long (self)	Yes
Long \times long	Yes
Long \times medium	Yes
Long \times short	Yes
Long \times rudimentary	Yes
Medium (self)	No
Medium \times long	No
Medium \times medium	No
Medium \times short	No
Medium \times rudimentary	No
Short/Rudimentary \times Long or medium or self	No

There was a clear spatial segregation of different styled flowers within an inflorescence (Figure 2). The flowers towards basal region were more often long styled, while rudimentary styled flowers were borne towards the upper portion of the inflorescence. The medium and short styled flowers were distributed in the middle region (Figure 2). Generally, the style length of the flowers reduced from bottom to the top of the inflorescence. Owing to acropetal blooming pattern, in any inflorescence long-styled flowers bloomed first followed by medium, short and rudimentary. The long-styled flowers constitute about 44% ($44.52 \pm 8.63\%$) of the total flowers in an inflorescence, medium $5.64 \pm 3.11\%$, short $19.54 \pm 4.80\%$ and rudimentary-styled flowers $30.63 \pm 7.98\%$.

The long-styled flowers produced mature fruits when pollinated with pollen derived from flower of any other stylar class as well as self pollen (Table 2). This indicates that the long-styled flowers are bisexual and pollen from all categories is fertile (Table 3). However, medium, short and rudimentary-styled flowers did not set fruits when they were pollinated from any other group or selfed hence function as males (Table 2). No differences

Table 3. Pollen size (value $\times 50$ microns), fertility (per cent) and ovule number (per flower) in different categories of flowers in *Caesalpinia*

Flower type	Yellow morph		n	Red morph	
	Sample size	Mean \pm SE		Mean \pm SE	
<i>Pollen size</i>					
1. Long	31	17.516 \pm 0.10	40	20.525 \pm 0.09	
2. Medium	36	18.306 \pm 0.17	22	20.318 \pm 0.23	
3. Short	42	18.571 \pm 0.17	38	17.237 \pm 0.13	
4. Rudimentary	40	18.425 \pm 0.11	43	20.302 \pm 0.10	
<i>Pollen fertility</i>					
1. Long	31	100 \pm 0.00	40	100 \pm 0.00	
2. Medium	36	100 \pm 0.00	22	100 \pm 0.00	
3. Short	42	100 \pm 0.00	38	100 \pm 0.00	
4. Rudimentary	40	100 \pm 0.00	43	100 \pm 0.00	
<i>Ovule number per flower</i>					
1. Long	44	7.886 \pm 0.07	35	7.914 \pm 0.15	
2. Medium	19	7.842 \pm 0.16	15	7.867 \pm 0.17	
3. Short	33	8.121 \pm 0.10	32	7.938 \pm 0.12	
4. Rudimentary	25	7.600 \pm 0.14	29	7.724 \pm 0.14	

'*t*' test non-significant.

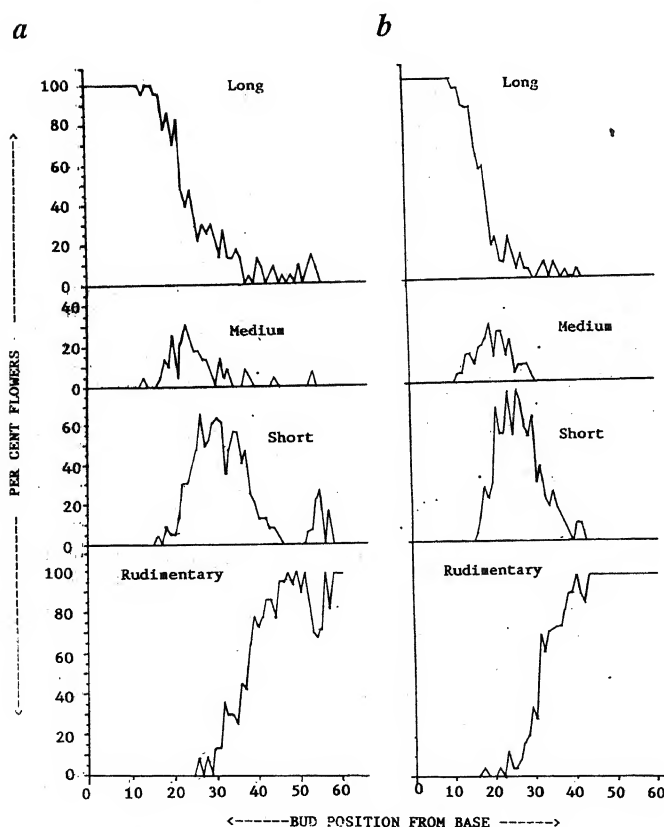


Figure 2 a, b. Spatial segregation of flowers with different stylar lengths within an inflorescence. *a*, Yellow morph and *b*, Red morph.

were observed in pollen size and fertility and ovule number in respect of different categories of flowers (Table 3).

Table 4. Dry weight (per flower basis including the stalk; in mg) of different categories of flowers in *Caesalpinia*

Flower type	Yellow		Red	
	<i>n</i>	Mean \pm SE	<i>n</i>	Mean \pm SE
Long styled	21	73.29 ^a \pm 5.136	27	84.67 ^a \pm 3.808
Medium styled	9	71.22 ^a \pm 6.144	28	70.57 ^b \pm 2.812
Short styled	20	56.55 ^b \pm 4.867	37	69.35 ^b \pm 2.642
Rudimentary styled	21	48.86 ^c \pm 3.152	29	51.93 ^c \pm 3.452

Mean values with same superscript (within a morph) do not differ statistically but with different superscripts differ statistically ('*t*' test significant $P < 0.001$).

These results show that in *Caesalpinia pulcherrima*, four morphologically distinguishable flowers based on stylar length are produced within primary inflorescences (Figure 1). Further, *Caesalpinia* plants produce nearly 45% flowers within an inflorescence which normally set into fruits while 55% flowers are functionally pollen donors (Table 3). Hermaphroditic flowers require higher levels of energy for their construct and maintenance and also require higher energy during pod-filling stage when compared to functional male flowers (Table 4).

Interestingly, it was observed that if the basal long-styled floral buds were either removed intentionally or predated, the subsequent medium and short styled floral buds reverted to long-styled flowers and set into fruits (personal observation). This suggests that failure of fruit set in categories like medium, short and rudimentary may largely be determined by post-fertilizational events. Infact, competition for limited resources among developing pods has been shown to be severe in *Caesal-*

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*pinia*¹¹. Flowers with rudimentary styles never set fruit, because they are devoid of a normal style and stigma, however, the function of the ovules in this category is not known.

Caesalpinia produces energetically expensive flowers during early stages of inflorescence growth while less energetic and less expensive medium and short styled flowers at later stages might act as a reserve for the possible risk of floral predation^{12,13}. This developmental plasticity of floral function mediated by energy constraints may also be adaptive in increasing pollination efficiency. Because flowers are retained on the inflorescence for more than two days after blooming, the display size of an inflorescence would increase substantially when terminal flowers in an inflorescence are in bloom. This would also increase pollinator visitation, hence the plant may gain more through pollen export in the later stages of inflorescence growth^{14,15}.

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Trondhjemite of the Alwar basin, Rajasthan: Implications of late Proterozoic rifting in the North Delhi Fold Belt

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Ajitgarh pluton, a minor granite–trondhjemite body, intrudes the Delhi metasediments of Alwar basin in the North Delhi Fold Belt (NDFB). The granite and trondhjemite, representing different pulses of anorogenic felsic magmatism, are distinct from the syntectonic granites of NDFB. The present work is also the first report of trondhjemite from Proterozoic environs of northwestern Indian peninsular region. Ajitgarh trondhjemite (AT) demonstrates trondhjemite mineralogy and chemical characters consistent with parameters prescribed for trondhjemite nomenclature. It is enriched in silica and depleted in CaO, MgO and FeO, as compared to the Archean TTG, and appears to be genetically related to the anorogenic magmatism of sodic affinity.

THE Trondhjemite–Tonalite–Granodiorite (TTG) association, a characteristic feature of the Archaean granite–

greenstone terranes, is hitherto unreported from the Proterozoic Delhi Super-group rocks from northern fringe of Indian peninsular shield. We report here the trondhjemite occurrence from a trondhjemite–granite suite from Ajitgarh pluton (27°26'N: 75°50'E), a minor intrusive body in the North Delhi Fold Belt.

Ajitgarh pluton, a composite granite–trondhjemite body, intrudes the sericite quartzite of Delhi Super-group. The geological set-up of the area is shown in the lithostratigraphic map (Figure 1). The contact between country rocks and the intrusive granitoids is obliterated by alluvial sand cover. Intrusive nature of the pluton is manifested by its emplacement discordant to the regional structural grain. Post-orogeny emplacement of granitoids is evident from absence of planar fabric, other textural characters and discordant nature of the pluton.

The generalized geological set-up of the area is summarized below:

Quartz–pegmatite veins
Granite
Trondhjemite
–Intrusive contact–
Sericite quartzite

The trondhjemite (AT), occupying the southeastern part of the pluton is in sharp contact with granite and

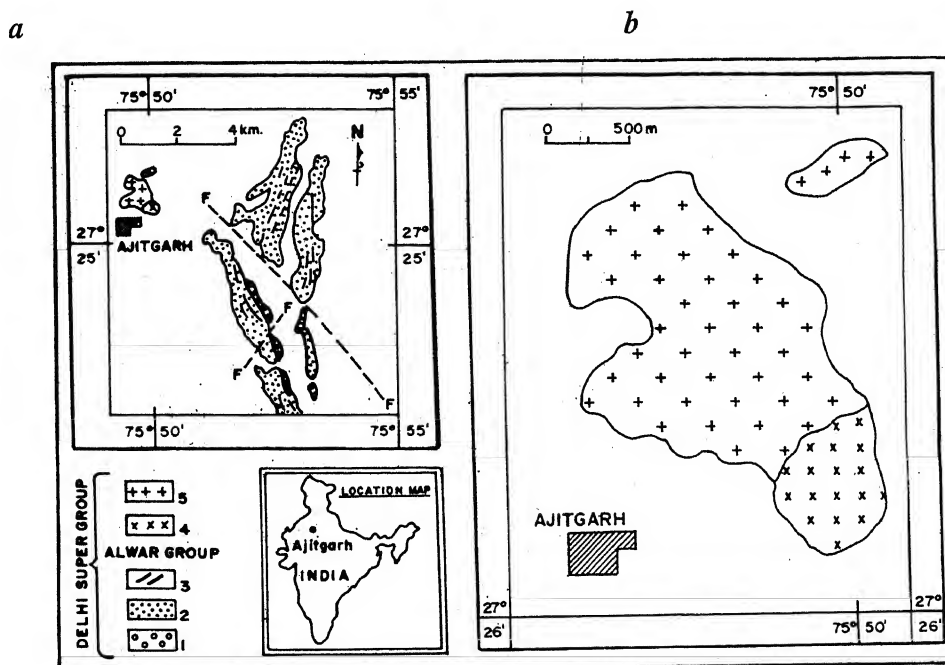


Figure 1. *a*, Map of Shahpura area showing regional lithological setting. *b*, Lithological map of Ajitgarh pluton showing distribution of trondhjemite and granite. Map of India showing location of Ajitgarh is also given in the inset. 1, Conglomerate; 2, Sericite quartzite; 3, Amphibolite; 4, Trondhjemite; 5, Granite.

Table 1. Modal composition of Ajitgarh trondhjemite

Sample no.	1	2	3	4	5	6	7	8	9	10
Quartz	39.5	38.0	43.0	45.5	47.0	44.5	40.0	47.5	44.5	45.5
Plagioclase	43.0	41.0	40.0	39.0	38.0	37.0	43.0	38.5	40.0	39.0
K-Felspar	12.0	9.5	11.0	10.0	10.0	11.5	10.5	8.5	12.0	11.0
Hb and Augite	3.5	8.5	4.5	1.0	2.5	3.5	2.5	3.0	2.0	1.5
Sphene	1.5	2.5	1.0	3.5	1.0	2.5	1.5	1.5	1.0	1.0
Opaque	0.2	—	0.5	0.5	2.0	0.5	2.0	1.5	0.5	1.5
Others	0.5	0.5	—	0.5	0.3	0.5	0.5	—	—	0.5

Hb, Hornblende; Others: Allanite, Zircon, Epidote.

represents an earlier magmatic phase. Both trondhjemite and granite are dissected by younger tourmaline pegmatite veins.

Ajitgarh trondhjemite (AT) is a medium to coarse-grained holocrystalline, non-foliated, massive and compact, leucocratic rock with predominant hypidiomorphic granular texture. Major mineral phases are quartz and plagioclase (oligoclase) with minor alkali feldspar, augite and hornblende. Sphene is the most abundant accessory mineral besides less significant zircon, epidote and allanite. Biotite is conspicuously absent.

Quartz, the most abundant mineral, is present as equant, anhedral grains. Plagioclase (oligoclase), ranging from An_{24} to An_{14} (determined by Michel-Levy method) shows limited compositional variation. The plagioclase laths show moderate sericitization and also

demonstrate replacement relationship with quartz. Augite ($Z \wedge C = 24^\circ$), occurring as anhedral grains, invariably shows replacive relationship with hornblende. Subhedral hornblende grains are characterized by strong pleochroism in shades of dark bluish green to pale green ($Z > Y > X$). In majority of cases, hornblende shows perfect reaction texture with augite where it is seen mantling the latter. K-feldspar is mainly microcline microperthite with a few grains of orthoclase. Sphene is present both as rhombic crystals and as anhedral grains. In the hornblende free varieties, it is the only coloured mineral noticed. In majority of cases it appears to be a late crystallizing phase. Well developed zircon crystals are usually present as inclusions within larger plagioclase laths. Epidote, allanite and opaque are the other minerals noticed.

RESEARCH COMMUNICATIONS

Table 2. Major element data for Ajitgarh trondhjemites and (CIPW) normative mineral composition

Sample no.	1	2	3	4	5	6	7	8	9	10
SiO ₂	75.02	73.84	73.27	76.47	78.65	77.45	75.74	78.04	78.32	78.15
TiO ₂	0.37	0.34	0.38	0.30	0.34	0.29	0.41	0.35	0.35	0.35
Al ₂ O ₃	12.05	12.56	12.96	12.21	12.17	12.23	12.60	12.23	12.38	12.48
Fe ₂ O ₃	0.46	0.59	0.85	0.43	0.13	0.26	0.47	0.36	0.24	0.39
FeO	1.15	1.43	1.97	1.04	0.31	0.66	1.66	0.88	0.57	0.99
MnO	0.04	0.04	0.05	0.03	0.02	0.03	0.03	0.04	0.03	0.02
MgO	0.54	0.53	0.34	0.20	0.32	0.24	0.25	0.10	0.09	0.08
CaO	1.51	1.36	1.36	0.53	0.62	0.72	0.92	0.59	0.58	0.55
Na ₂ O	4.80	4.40	3.96	4.11	4.24	4.01	4.60	4.08	4.42	4.63
K ₂ O	1.66	1.59	1.43	1.51	1.58	1.53	1.38	1.50	1.53	1.51
P ₂ O ₅	—	—	—	—	—	—	—	—	—	—
	97.60	98.68	96.57	96.83	98.38	97.42	97.56	98.17	98.51	99.15

CIPW Norms

Q	36.40	37.70	40.26	45.06	47.48	46.56	40.85	47.36	46.42	44.26
Or	9.81	9.40	8.45	8.92	9.34	9.04	8.16	8.86	9.04	8.95
Ab	40.62	37.23	33.51	34.78	35.88	33.93	38.92	34.52	37.40	39.18
An	6.95	6.75	6.75	2.13	0.38	2.53	4.56	1.93	0.30	2.23
C	0.41	1.13	2.43	3.03	3.45	3.05	1.89	3.19	3.31	2.23
Hy	2.54	2.97	3.23	1.61	0.26	1.17	1.74	0.81	0.35	0.36
Mt	0.67	0.86	1.23	0.62	0.10	0.38	0.68	0.52	0.35	0.28
Il	0.70	0.65	0.72	0.57	0.65	0.55	0.78	0.66	0.66	0.49

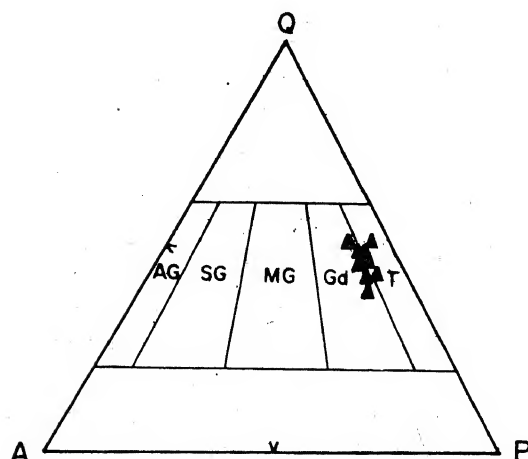


Figure 2. Modal QAP diagram of Ajitgarh trondhjemites. T, Tonalite; Gd, Granodiorite; MG, Monzogranite; SG, Syenogranite and AG, Alkali granite.

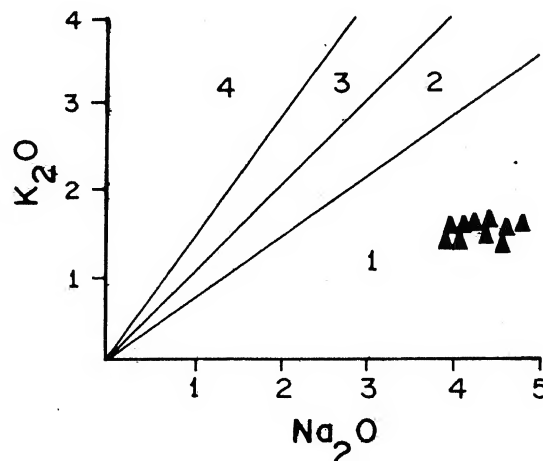


Figure 3. Na₂O-K₂O diagram showing tonalitic character of AT. 1, Tonalite; 2, Granodiorite; 3, Adamellite; 4, Granite.

Table 3. Comparison of AT with trondhjemite values

	Trondhjemites ¹	Ajitgarh trondhjemite (Present data)
SiO ₂	68–75%	73.27–78.65%
Al ₂ O ₃	<14% at 75% SiO ₂	12.07–12.96%
FeO(t) + MgO	<3.4	0.75–3.08
Na ₂ O	4–5.5%	3.96–4.80%
K ₂ O	<2%	1.38–1.66%
CaO	1.5–3%	0.53–1.51%

Paragenetic relationships indicate initial plagioclase–pyroxene crystallization, followed by separation of quartz and hornblende. Sphene and epidote mark the terminal phase of crystallization. The textural characters do not suggest any deformation as evident by equant nature of quartz grains, absence of preferred mineral orientations and absence of recrystallization.

Modal mineralogical composition of AT shows predominance of quartz and plagioclase (together constituting more than 80–85% of total rock volume) over other

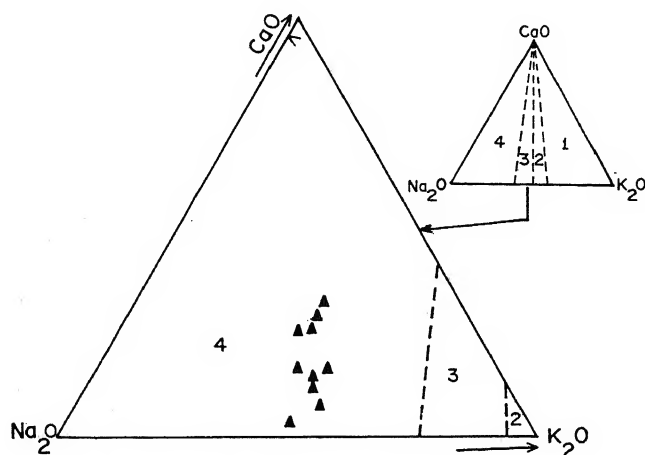


Figure 4. Na_2O - K_2O - CaO diagram showing tonalitic character of AT. 1, Granite; 2, Quartz monzonite; 3, Granodiorite; 4, Tonalite.

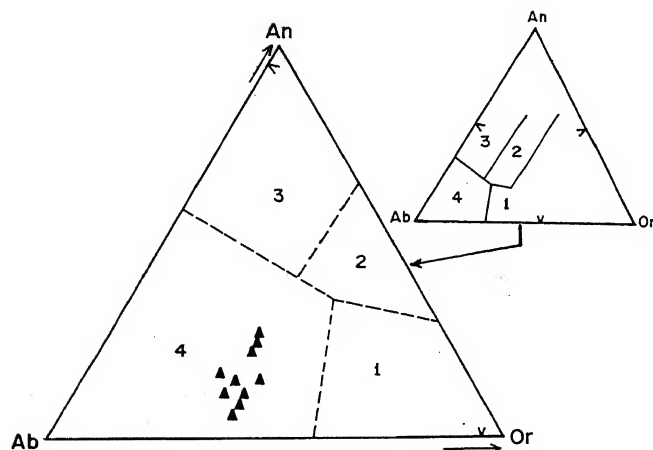


Figure 5. Normative Ab-An-Or diagram showing trondhjemitic nature of AT. 1, Granite; 2, Granodiorite; 3, Tonalite; 4, Trondhjemite.

minerals (Table 1). K-felspar concentration is about 10% whereas augite and hornblende, together show minor variation from 1 to 4.5% (with one sample having a high value of 8.5%). Sphene concentration is quite consistent, varying from 0.5 to 3%. In the modal Q-A-P diagram the AT plot in the tonalite-granodiorite fields (Figure 2).

The trondhjemitic character of AT is further substantiated by chemical parameters. The major element data on ten representative AT samples are given in Table 2. Chemical homogeneity indicates their cogenetic nature. High $\text{Na}_2\text{O}/\text{K}_2\text{O}$ ratio (2.68 to 3.33) underlines sodic affinity. Appearance of normative corundum is attributed to low CaO and K_2O and does not indicate any alumina over saturation. Major element data for AT are within the prescribed ranges for trondhjemite and elemental abundances are coherent with typical trondhjemite values¹ (Table 3).

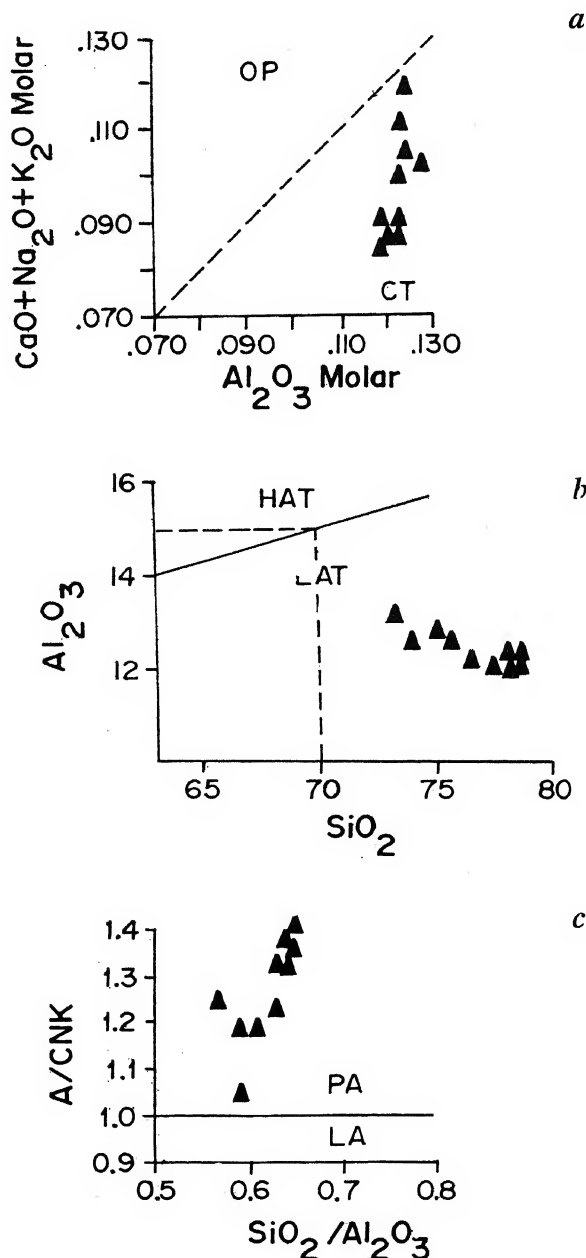


Figure 6a-c. a, Molar A-CNK diagram showing continental trondhjemitic affinity of AT. CT, Continental Trondhjemite; OP, Oceanic Plagiogranites; b, SiO_2 - Al_2O_3 diagram showing low alumina nature of AT. HAT, High Alumina Trondhjemite; LAT, Low Alumina Trondhjemite; c, A/CNK- $\text{SiO}_2/\text{Al}_2\text{O}_3$ diagram. PA, Peraluminous; LA, Low aluminous.

The AT shows slightly higher silica and lower CaO than prescribed for trondhjemitic rocks. The highest CaO value of 1.51 is at par with the lower limit for trondhjemite. An FeO/MgO ratio of 2 to 3 has been suggested for trondhjemite. In the present case the ratio varies from 1.33 to 12.73, with majority ranging from 2.54 to 5.06.

Alkali ratio has been a significant parameter in granitoid nomenclature and felsic rocks with sodic affinity

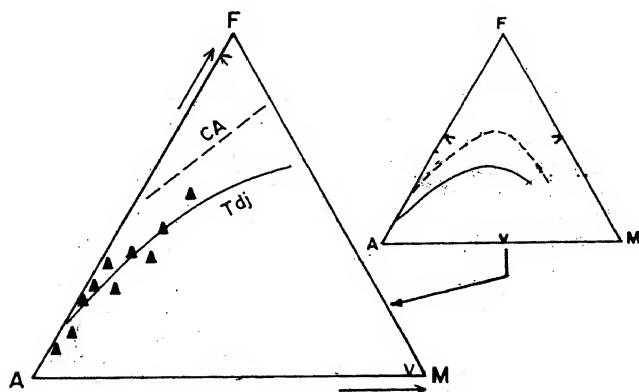


Figure 7. AFM diagram showing trondhjemitic trend.

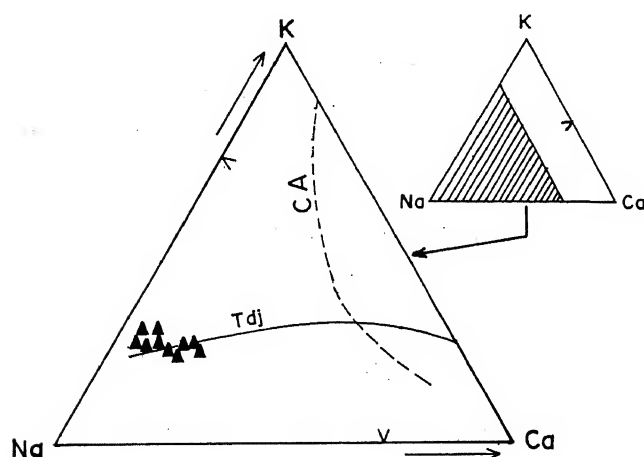


Figure 8. Na-K-Ca diagram showing trondhjemitic differentiation trend for AT. Tdj, Trondjemite; CA, Calc-alkaline.

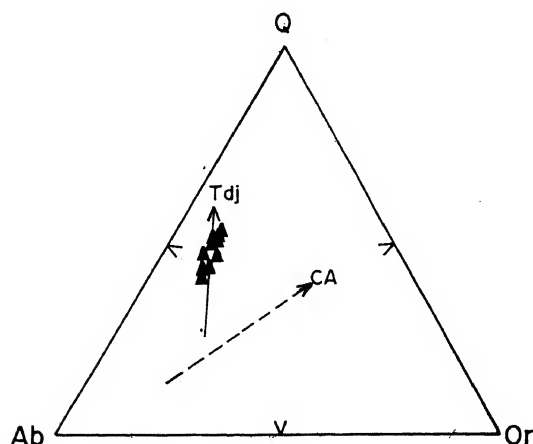


Figure 9. Normative Ab-Q-Or diagram showing trondhjemitic trend for AT. Tdj, trondjemite; CA, calc-alkaline.

($\text{Na}_2\text{O}/\text{K}_2\text{O} > 2$) have been named as tonalite². In the $\text{Na}_2\text{O}-\text{K}_2\text{O}$ and $\text{Na}_2\text{O}-\text{K}_2\text{O}-\text{CaO}$ diagrams³, AT plot in the tonalite field (Figures 3 and 4). In the normative

Ab-Or-An diagram¹ also, the AT shows discrimination with tonalite and unambiguously plot in the trondhjemitic field (Figure 5). Continental trondhjemitic affinity of AT is reflected in A/CNK ratio of > 1 . In the molar A-CNK diagram (Figure 6), these plot in the continental trondhjemitic field, quite distinct from oceanic plagiogranites⁴. In the A/CNK- $\text{SiO}_2/\text{Al}_2\text{O}_3$ diagram, these plot in the peraluminous field. In the $\text{Al}_2\text{O}_3-\text{SiO}_2$ diagram these plot in the Low Alumina Trondhjemitic field. The fields, proposed for a silica range up to 70% have been extended to accommodate silica-rich AT (Figure 6).

Trondhjemitic fractionation trend of AT is faithfully reflected in various sensitive discrimination schemes. In the AFM diagram, the AT plot along trondhjemitic trend (Figure 7). The distinction between classical calc-alkaline and trondhjemitic trends is also seen in Na-K-Ca and normative Ab-Q-Or diagrams (Figures 8 and 9) wherein the AT defines trondhjemitic path of descent.

Thus the mineralogical composition and chemical signatures establish the AT to be continental trondjemite, *sensu stricto*.

Trondjemites are characterized by moderate CaO, MgO and FeO for a given value of silica. A comparison of AT with Archaean trondjemites underlines broad chemical similarity although minor deviations are discernible. The AT are relatively enriched in silica and depleted in CaO, MgO and FeO, however, the alkali abundances are comparable. The average CaO abundance of 0.82% (range 0.53 to 1.51%) for AT is much below the usual values for Archaean TTG. However, a CaO value below 1% is not uncommon in Archaean TTG and CaO concentration as low as 0.29% has also been reported⁵. Relatively higher A/CNK for AT can be attributed to low CaO. The plagioclase in Archaean TTG is usually of oligoclase-andesine variety and in the present case the plagioclase (oligoclase) composition varies from An_{24} to An_{14} . Absence of biotite is a characteristic feature of AT. Minor biotite is usually present in Archaean TTG though pyroxene and amphiboles as the main mafic phases have also been reported.

Each felsic pluton has a distinct source and unique petrogenetic history, constraining any generalization. The difference between AT and Archaean TTG can be attributed to their distinct tectonic environments and evolutionary trends.

A 170 km long NNE-SSW trending lineament (extending between Khetri and Dudu), the 'albitite line' of Ray⁶, represents a post orogeny rejuvenation of the rift system that controlled Delhi sedimentation. This intraplate magmatism has tentatively been correlated with 800 Ma anorogenic event⁷. Instead of a single 'albitite line' the intrusion seems to be along a number of subparallel fractures⁸. The lineament passes to the west of Ajitgarh pluton in close proximity. Post-orogenic emplacement of AT, its anorogenic tectonic

environment⁹, sodic affinity and close proximity with intraplate sodic magmatism (albitite line) suggest a possible genetic link between two events. The relationship needs to be understood through detailed trace element and isotopic data.

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BOOK REVIEW

Quantum Biology by S. P. Gupta. New Age International Pvt. Ltd., 4835/24, Ansari Road, Daryaganj, New Delhi 110 002. 1996. 102pp. Rs. 250.

There is a never-ending demand for specialized text books, which can be easily accessible to research workers and post-graduate students. Although quantum biology is not a very new subject, the emphasis on use of different methods has changed from time to time. This was mainly due to progress in the computer hardware and availability of software at a reasonable price. Thus in the sixties people had access to 16 or 32-bit computers like IBM 1600, Honeywell 400 M, etc. and use of nonhomogeneous empirical methods as Del-Re method for σ and Huckel method for π electrons were popular. The most popular books were *Quantum Biochemistry* by B. Pullman and A. Pullman (Inter Science, New York, 1963) and *Electronic Aspects of Biochemistry* (ed. B. Pullman, Academic Press, 1964). In late sixties and early seventies, methods based on Self Consistent Field (SCF) theory approach with neglect of differential overlap as: Parriser-Parr-Pople (PPP), CNDO, INDO, etc. were developed and became popular because of availability of IBM 360, CDC 3200 and UNIVAC 1400 computers. The books *Approximate Molecular Orbital Theory* (J. A. Pople and D. L. Beveridge, McGraw Hill, 1970), *The World of Quantum Chemis-*

try (eds R. Daudel and B. Pullman, D. Reidel, Dordrecht, 1974) were found to be extremely useful. Much of the information was still available in good review articles like 'Organic molecule studies by semiempirical method', by Fonnandez-Alonso. The applications of biology were covered in books by Kier like *Quantum Pharmacology* (Academic Press) or *Molecular Orbital Theory in Drug Research*. Application of *ab initio* methods became popular with the availability of better IBM and CDC machines and computer programs through quantum chemical program exchange (QCPE) at University of Bloomington, Indiana, USA. There had been several short reports and even a dictionary of *ab initio* charges by Richardson was available. There had also been some specialized books like *Chemical Applications of Atomic and Molecular Electro-static Potential Molecular Electrostatic Potentials* by P. Politzer and D. C. Truhlar (Plenum Press, 1981).

Today the most important thing is to select the right method for a particular problem since lot of software is already available from commercial vendors as Biosym, Tripos Asso, Gaussian Inc. and also freely supplied as a public domain software. Hardware prices are reduced and their speeds have increased fantastically. The main difficulty for a researcher, especially of a biology origin, is to have training enough to carry out his work. Most of the time, the user is not interested in development of hard core theory or writing his own software.

He just has to know essential details of the methods and use in particular cases so that he can just pick up the method and do calculations. This demands more concise books which should be easy to follow. S. P. Gupta's book represents an ideal book for this purpose. It is concise and gives basic principle of ZDO approximation. He has enumerated essential details of CNDO, CNDO/1, CNDO/2, INDO, NDDU, PCILO, EHT, HMO, Del-Re, and Pariser-Paar-Pople method. He has given use of these methods for nucleic acids, proteins and phospholipids. He has also given a chapter on the use of these methods for quantum mechanics and transport in biological systems and drug-biomolecule interaction. The book is a perfect textbook for post-graduate and post-doctoral students in biomedical sciences. I only felt that the author could have given some information on *ab initio* method and molecular electrostatic potential maps, using different MO methods, their applications and some information on software packages. That would have benefitted the reader. The book on the whole is well written. The author and publisher have taken great efforts to set the mathematical equations.

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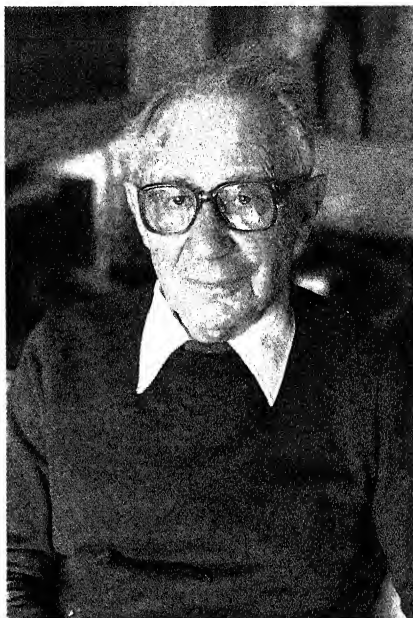
Tadeus Reichstein (1897–1996) – An obituary

Tadeus Reichstein, Nobel Laureate and Foreign Fellow of the Indian Academy of Sciences, passed away on August 1, 1996 at the age of 99 years.

T. Reichstein was born on July 20, 1897 at Wloclawek, Poland. Due to unsettled conditions in Eastern Europe, he emigrated initially to Berlin and later to Zurich where he was granted the Swiss citizenship in 1914. Reichstein studied at the Zurich Technical University, graduating with a degree in Chemical Engineering in 1920. For his work on the odiferous constituents of coffee under the supervision of Hermann Staudinger, he was awarded the doctorate degree. This work formed the basis for the production of powdered coffee.

Reichstein joined the Zurich Technical University as a lecturer in 1930 in the new research specialty of physiological chemistry. He was promoted to assistant professor in 1934 and associate professor in 1937. During this period he worked on the structure and synthesis of carbohydrates, a field which he pioneered, especially in the crystallization of carbohydrates. During these years, his work on the synthesis of vitamin C involving an oxidation

process is noteworthy and several thousand tons of this vitamin are manufactured worldwide using his process. In



1938, he moved to Basel University as the head of the pharmacy department where he did the pioneering work on the isolation, structure, partial synthesis and biological effects of the adrenal cortex hormones and also cardiac

glycosides. Several corticosteroids were named after him. In 1950, he shared the Nobel prize for Physiology and Medicine for this work along with P. S. Hench and E. C. Kendall.

In 1950, Reichstein became the first director of the world renowned Institute of Organic Chemistry at Basel and retired in 1967. During the last 25 years, Reichstein became interested in chemotaxonomy and was responsible for the classification of ferns.

Reichstein received many honours and awards, the most important being the Royal Society's Coplay Medal (1968) besides the Nobel Prize for Physiology and Medicine. The Swiss Society of Pharmaceutical Sciences created the Reichstein Medal for excellence in pharmaceutical sciences in honour of Reichstein.

Reichstein had many Indian collaborators, most notably S. Rangaswami. Tadeus Reichstein married Lousie von Ufford in 1927, and is survived by a daughter.

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Chandrasekhar – some reminiscences*

S. Ramaseshan

Introduction

Friends,

I consider it a great honour that I have been asked to deliver the bicentennial lecture of your historic Institute. For me the Indian Institute of Astrophysics has always been the home of Indian Optical Astronomy. It has been in the vanguard of building optical telescopes for India.

I first went to Kodaikanal when I was eight years old. The Kodaikanal solar telescope was the first telescope I had ever seen. I shall never forget the excitement I felt. Nor the thrill I got when I saw for the first time sunspots projected on a screen. I have visited Kodaikanal several times later. The old clocks of IIA at Kodaikanal have to be seen to be believed. The renovated Evershed hall – commemorating a great discovery – is beautiful. The 2.3 m telescope at Kavalur is a magnificent achievement. I now understand that the entire Institute is united in pursuing the goal of setting up a two-metre telescope and possibly a much larger one in Hanle or Ladhak which may prove to be one of the best telescope sites in the world. I wish you all luck. All this warms my heart. For I was closely associated with your Institute when it was formally formed; when it moved to Bangalore; and when the money for the Kavalur telescope, the dream of Vainu Bappu, was sanctioned. I feel quite embarrassed by the way I have been introduced by Prof. Cowsik, today, and more so with what is written in the brochure. He must have done much archaeological digging about me and my science.

Some time ago Cowsik requested me to give this bicentennial lecture. He said, 'It will be befitting if you talk about S. Chandrasekhar this year. He

was an Honorary Fellow of this Institute'. I too felt that Chandrasekhar would be an appropriate topic – as he was associated with an Optical Astronomical Laboratory – Yerkes – for almost 25 years. Cowsik requested me so earnestly that I should not say NO.

This reminds me of a cartoon in *New Yorker*. A middle aged lady is pictured surrounded by children of differing ages and of various nationalities, Japanese, Indian, Arab, Caucasian, African and so on. The legend says – 'she worked for the foreign service. Her problem was she could not say NO'.

I now regret having accepted this invitation. I was wondering what I should talk about – Chandrasekhar's science – How can anyone cover it in one hour even if one were competent to do so. There is Rajaram Nityananda's talk at IPA Madras entitled 'The six faces of Subrahmanyan Chandrasekhar' punning on the belief that God Subrahmanya is a six-faced god.

Should I talk about the great human being he was? Wali has done this in 325 pages. Then there are these wonderful articles by Abhay Ashtekar, G. Srinivasan and others again in *Current Science* – which I presume you have read – if not you must. I must be pardoned for mentioning *Current Science* again and again. For this has been my obsession for the last 6 or 7 years. Sometime ago I heard Radhakrishnan telling someone – 'I have known Ramaseshan for almost 60 years – we always discussed every subject under the sun – Optics and Astronomy, Physics and Aerodynamics, Cars and Gadgets, Planes and Gliders, Materials and devices, etc. etc., we have had a great deal of fun together. But now I find it a little difficult to talk to him. For instance if I tell him I saw a very pretty girl – so strikingly beautiful that it may even make his heart flutter.' Ramaseshan replies: 'That is very nice – can you write it up for *Current Science*'.

I can therefore talk to you only about my personal interactions with Chandrasekhar. That is why I gave the present title to this talk. Here again I

have a problem. Since he first left India in 1930 I have met him many times and after each meeting I have come back exhilarated. I have four files of letters we have exchanged. And providence has showered on me an unexpected gift – we became very good friends.

'I should not be writing this letter' he wrote 'if I were not sure of your long standing friendship'.

Even today this appears to me to be unbelievable. You can therefore understand my reluctance to wash linen in public even if it is not dirty. I fear also I may commit the cardinal error of making myself a central player. For what is Chandrasekhar's stature and where am I?

I have taken seven or eight incidents from my large collection which may possibly interest you and have prepared this talk. Three things I have to say. The incidents I relate are essentially correct. But memory can be deceptive. When I use the active voice, the words might not be the exact ones spoken at that time. Finally I lace the stories with my comments which you may ignore if you like.

The last conversation and his death

My last conversation with him was in July 1995. I called him up from Canada on my way to Montreal. We talked of many things for a long time. He said he had finally received the printed copy of his book on Newton's *Principia* – 'I like the cover. It is magnificent'. He had worked on this book for almost 5 years. 'My work is finished' he said. Did he see the figure of the Grim Reaper close by. On hearing that I was not going to Chicago due to reasons of my health, he asked almost in a wailing voice – 'Sivaraj (that is me) aren't you coming to see me?'. That question still haunts me – for within two months he was dead.

I was quite ill, when in the early morning of August 22 I got a telephone

*A verbatim account of the bicentennial lecture given at the Indian Institute of Astrophysics, Bangalore on March 14, 1996. Reproduced from *Bull. Astron. Soc. India*, 1996, 24, 537–550.

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call telling me that he was no more – it was a few hours after his death on 21st in Chicago. I was calm – I phoned a few friends in USA. It appears he had a slight pain in the chest on the 19th morning. As it was on the right side he and Lalitha were not too worried. In fact so unworried that they even discussed, I am told, what they should do on the occasion of their 60th wedding anniversary which was to come a year later in September 1996. He phoned his sister who was in USA that he was preparing a series of lectures to be given at various universities. He therefore requested her to postpone her visit to Chicago by a week – he obviously died in harness.

On 21st morning after breakfast he drove to the University Clinic to get himself checked. Doctors told him that he was having a massive heart attack and were horrified that he had driven to the clinic himself. His car was not parked in the regular parking area but in the emergency one. One guesses that he had the heart attack during the drive. They phoned Lalitha. She came and was with him for 10 minutes. He was heard to tell her 'I think it is time for you to start making the preparations we had planned'. He died sometime later. It is almost certain he did not suffer much. He had stipulated that there should be no memorial service or meeting. He was to be cremated and no one was to be present and the ashes should be brought to Lalitha. As Plato says of his master the great Socrates, I quote:

Such was the end of a man who I think was the wisest and the justest and the best man I have ever known.

I was calm and collected. I told myself he was almost 85, he had become a legend in his time and was the greatest astrophysicist of this century. He had chosen perceptively fields which were seemingly unfashionable which later were to blaze new trails. He had produced 50 or more remarkable students who were dotted all round the world. He had written essays on science, literature, and the arts which were master-pieces. He had said to me – my work is finished and it seemed to me as though he had arranged with Providence for his passing away.

When I was invited to Montreal to speak at the memorial meeting for Dorothy Hodgkin (who also considered me a friend) – Kausalya, my wife, said

that on no account must I break down and cry before a western audience.

Today she told me: you have a good Indian audience, familiar with Kannada, Tamil and Hindi films: you could therefore break down and cry, if you wish!

Polarization of the sunlit sky

I met him first when he came to Calcutta to bid goodbye to my mother on the eve of his going to England in 1930. I was then less than seven. Again in Calcutta when I was 12 years old, I heard his lecture at the Indian Association for the Cultivation of Science. He wore the traditional cylindrical dhoti and a coat which was to become his standard dress whenever he was in India. I still remember him mentioning a mnemonic used for classification of stars. O BE A FINE GIRL AND KISS ME RIGHT NOW SALLY.

Fifteen years later in 1951 when he came to Bangalore he was received at the airport by C. V. Raman. He came to lunch at our little house at the Indian Institute of Science, I was recently married. He was full of laughter, made fun of us, quipped with Kausalya and discussed with her sari fashions.

I think it was then that he told us of the experiences he had in Russia. The following story he told us is the same he also related at a party in his house in Chicago.

He was shocked when he had to share a small cabin with three women in a boat during his North Sea passage to Russia. The women too were horrified. They developed an elaborate ritual for undressing so that the man would have no opportunity of seeing them undressed. They all undressed and went to bed. The women insisted on keeping the little port hole window open so as not to make the cabin too private! North Sea became quite rough as it normally does; even then the women refused to permit Chandrasekhar to close the port hole. Finally a very big wave came and splashed into the cabin through the port hole and drenched them; all jumped out of their beds in a state of undress. When Chandrasekhar told this story everyone at the party laughed. There was a woman post doc who said, 'Are we to take it Chandra that you had a shower with three undressed women at the same time – it was very very naughty of you'. This provoked an immense roar of laughter.

In many of the stories that Chandrasekhar told he was often the target of the joke*.

He told us how he had met a large number of brilliant astronomers and physicists in Russia. All of them were later sent to Siberia, purged or executed. Landau and Ambartsumian were the exceptions – thanks to the heroic intervention of Peter Kapitza.

The next day he gave a lecture 'On the polarization of the sunlit sky'. It was perhaps the best scientific lecture I have ever heard. It was the pinnacle of his monumental work on Radiative Transfer. He told us how he got interested in this problem – the problem of the atmosphere of a star. The need to treat coupling by photons of the different layers with varying properties. He told us how the principle of invariance was evolved – following the work of the Armenian astrophysicist Ambartsumian I referred to earlier (in Russia from his first name in the passport he was often taken for an Armenian as it had the same ending – Subrahmanyan – Ambartsumian) – He developed 'invariant embedding'. This he applied to explain the polarization of skylight – the radiative transfer in a finite slab of air which scatters the sunlight in the sky. He went back to the Great Masters, he discovered Stokes' paper published in late 1870s from which he obtained the Stokes parameters for the description of polarized light. He formulated integral equations which were so complex that even very good mathematicians would have abandoned them. To quote Rajaram Nityananda 'He talked to these equations personally and intimately till they gave up their secrets'. It is a saga that has to be heard or read to be believed. At the end of the lecture Raman who was in the audience commented 'Rayleigh formulated the theory of light scattering to explain the blue of the sky in 1871. For eighty years the problem of the experimentally observed complex polarization of skylight has defied the efforts of the great masters. Today we have had a most extraordinary exposition by another Master. We have heard how the problem was solved by the combination of physical intuition and powerful mathematical analysis.' Then he asked: 'Professor Chandrasekhar can you tell us how long it took you to work out the results which you have pub-

* I have heard many versions of this story but this is the one I like best.

lished in the four-page paper in *Nature*?. Chandrasekhar after some thought answered 'More than two and a half years'. That issue of *Nature* (1951) had not reached Bangalore then. But Chandrasekhar, as was his habit, had sent a reprint or preprint by airmail to Raman.

One realized that if one had some previous knowledge of the subject (as I had on polarization of light and the Stokes parameters, etc.) one can get much more from an outstanding lecture by one like Chandrasekhar. But the awful disadvantage is that such a lecture exposes one's inadequacy unforgettingly.

I jump 35 years – as this relates to another Indian who was also an expert in the same field. Chandrasekhar once wrote to me:

Stimulated by the handsome references to Pancharatnam by Michael Berry, my colleagues and I are interested in the *Collected Works of Pancharatnam*, I should like to buy a copy.

I sent him a complimentary copy of the *Collected Works*. As the High Priest of Polarization Optics of this century I asked him whether he would be inclined to write for the Pancharatnam issue of *Current Science*. He wrote,

I am too far away from this subject – It is almost 45 years since I even thought of Polarization of Light. Further I may not have anything worthwhile to say. However I always congratulate myself that when I read Pancharatnam's classic paper long ago I did recognize its merits.

Time does not permit me to talk to you about my meeting him in Yerkes in 1954; his accounts of his collaboration with Enrico Fermi, Fermi's death which had occurred just a few weeks earlier (He told me the well-known story of the elephant which all of you must have heard). How he decided and took the US citizenship in 1953 (without consulting his father). 'Now Lalitha has been appointed as the peace maker' and he told me of her efforts to appease the old man. I remarked 'You coward'. He told stories of his and Raman's encounter with colour prejudice in USA, his introducing me to Otto Struve, whom Blaauw described as the man who knew all of astrophysics, and many other stories. I shall never forget this meeting.

Einstein's view of God

I mentioned that he and I had exchanged many letters. I give an example which illustrates how meticulous he was in answering questions asked of him, the effort he takes to make his listeners understand, his consideration and his strong views.

'I am writing this letter, however, with respect to two questions you asked of me at the airport prior to our leaving Bangalore a week ago'.

First, with regard to Einstein's different views of God: in 1919 when he said 'I would be sorry for the Lord' and his later statement in the late twenties 'God does not play dice'.

Chandrasekhar was always uncomfortable when the word God was mentioned. The letter contains a few typed pages and has some equations. His argument is that the same metric (using the post-Newtonian approximation) gives the precession of the perihelion of mercury to be 43 seconds of arc per century and the deflection of light grazing the Sun to be 1.75 seconds. A theory which is consistent with one crucial test, if it fails in another – then one would be justified in being sorry for the "Lord". 'Ignoring the word 'Lord' I do not think anyone would disagree with what Einstein intended to say.'

'Your second question really concerns the spin of the photon. That the photon must be associated with an angular momentum $s = 1$ which is as old as Einstein's concept of the photon (remember the weight 2 in Planck's formula).' Then a long discussion follows on Bohr's correspondence principle, Rubinovicz' derivation, the demonstration of the spin of the electron by the Mottdouble scattering experiments and the need for explicitly demonstrating the spin of the photon etc., etc. He ends the letter by saying,

I hope you will not consider me too impertinent, if I quoted Eddington in this context. In the off chance that posterity may find wisdom in our words is no reason for making meaningless noises.

In another context, he wrote: 'Einstein's judgment on the uncertainty principle is one of personal conviction only. It is not any reasoned scientific argument. His later official judgement on the quantum theory does not invalidate his own personal conviction of the truth of

general relativity.'

Wali's biography and Raman

He asked me once what I thought of Wali's biography.

'It is a remarkable biography. It reads like a novel'.

'Do I detect a slight undertone of disapproval' he asked.

'Yes, I do not like those parts where by implication it is derogatory to you'.

'Derogatory to ME' – the Me became a high monotone – reminding me of his uncle. I think he could not believe, that Wali his Votary, could have said anything derogatory to him.

'When Raman offered you a position at the Indian Institute of Science in 1935 – you wrote to him a very fine courteous and what I consider to be an honest letter; that you were flattered, you were not sure whether you would fit into an experimental group and finally you were afraid that the media attack against Raman would increase if you, his nephew, were appointed. And this may affect your performance as a scientist. Therefore you were, with great regret, not accepting his offer.' Wali, on the other hand, has written it in such a way that one gets an impression that you were really responding to your father's request to stay away from Raman. This implies the letter was a false one – that you were dissimulating. I have now known you for a long time. I cannot imagine you dissimulating or playing the hypocrite.

Chandrasekhar was silent for some time and said with a smile,

I did not write the biography it was Wali who did.

Chandrasekhar was always reluctant to talk about Raman. I once cornered him and made him speak on this topic.

In your letter when Lalitha and you presented Ramanujan's bust you say:

As a companion to the bust of Raman so that the bust of the greatest physicist of India could be along with that of the greatest mathematical genius of our times who happened to be an Indian

'Do you really think Raman was the greatest physicist of India?'

'Of course I have no doubt on that

score – and you yourself have sometime ago given me a certificate that I do not dissimulate.'

People in India remember him for the discovery of the Raman Effect which won him the Nobel Prize. One discovery advances science, but it cannot be a criterion to judge the quality of a physicist. Raman's work on acoustics is first rate – his work on the violin is considered a classic even today. Acousticians wonder how he could have done these beautiful experiments and theories in those days. In optics he was a Master, in the class of Rayleigh and Michelson. It is a great pity his *Lectures in Physical Optics* which I used for giving an advanced course in Optics is not widely known.

His Nobel Prize, in my view, retarded his science – probably it was because the effect it had on his psychology, I may be wrong. Even so his later work on Brillouin Scattering and crystal transformations are again world class. One feels sorry he committed major errors in his latter life – and he became aggressive too. Unfortunately this is what many remember him by, not the excellent physics he did earlier. I consider him to be a brilliant classical physicist who made a spectacular experimental discovery of a quantum mechanical phenomenon. It has influenced physics, chemistry and even technology as he predicted in his Nobel Lecture.

Most of the other Indian physicists of repute, of those days, had practically no outstanding students. On an invitation from CSIR I had the opportunity of visiting a large number of laboratories and scientific institutions of India. I realized Raman's contribution to the growth of Indian Science and Technology, after India's independence, was also in the form of students. Many of them had established very competent research schools – many had headed and built laboratories. They must have imbibed from him the spirit of science for they, in turn, produced excellent students.

'How do you rate Raman by world standards?'

'As an experimental physicist'

'Yes'

'For that we have to compare him with men like Rutherford and Fermi – the answer is obvious'.

'If that is the yardstick you use would not all experimenters of the world except perhaps those like Michael

Faraday fall by the wayside?'

After some thought he remarked: 'Yes, that is probably true.'

He enters general relativity

The following story was told to me by Chandrasekhar himself. He wanted to go to Warsaw to the Conference on General Relativity which was to be attended by the established figures in the field like Richard Feynman, Leopold Infeld, Paul Dirac and the younger ones like Roger Penrose and Ted Newman. The National Science Foundation wanted to support his travel. He did not fit into the standard categories they had.

Q1 Are you chairing a session NO

Q2 Are you giving an invited talk? NO

Q3 Are you making a presentation? NO

Q4 Are you an expert? NO

I want to be there as I am seriously thinking of entering the field of General Relativity. They found an appropriate excuse to give him travel funds.

A friend of mine was visiting Chandrasekhar. They were walking down the corridor. Suddenly he saw someone coming at the end of the corridor. He was a young man, wearing dirty torn jeans. Chandrasekhar was of course immaculately dressed, in his charcoal grey suit, handsome as ever. He straightened up his tie, went forward at a fast pace, greeted the young man, shook his hand, talked to him for some time, opened the door of the car and the young man got in and drove off. Chandrasekhar came back and joined my friend who asked 'who is that hippie?'

'He is Geroch.'

'Who is Geroch?'

'He is one amongst the most outstanding relativists of today – he is in his twenties. I have invited him to lecture in Chicago this summer. I am attending his course. It takes me the entire weekend to work out and understand some of the equations he puts on the black board – to be ready for his Monday lecture. When I am stuck. I ring him up, even if it is late at night, to bail me out.'

At 60 he was humble enough to learn

relativity from youths, at 70 held his head high amongst the brilliant young relativists. At 80 he was respected by all for his unparalleled contributions to General Relativity.

It was about this time that Chandrasekhar told me

The trouble with Einstein was that he did not believe sufficiently in the Theory of General Relativity.

I honestly felt that he was going gaga. Many years later after he wrote his monumental treatise on Black Holes I told him of the fears I had. He laughed loudly in his characteristic way.

It was later when Abhay Ashtekar wrote the following that I understood what Chandrasekhar meant.

'If Einstein had believed in his general relativity sufficiently and deeply enough, he would have worked out the consequences of the nonlinear aspects of the theory in the strong field regime. Chandrasekhar believed that Einstein was the *only* person who could have done this at that time. Then General Relativity would have entered the mainstream of physics four decades earlier.'

Chandrasekhar told me that many authors had not considered the gravitation radiation reaction in General Relativity. 'When all my old problems were reexamined, very unexpected results turned up. For example I was able to show that all rotating stars are unstable due to radiation reaction.'

Abhay Ashtekar says:

Chandrasekhar more than anyone else played a decisive role in bringing the beautiful creation of Einstein to its natural home – Astrophysics, and

His intuitive feeling that the radiation reaction in general relativity is important is one of the deepest insights in relativistic astrophysics.

Music, literature and the arts

Chandrasekhar's appreciation of Music, Literature and the Arts was staggering. He mastered each of these fields in his own way. He heard each of the symphonies of Beethoven and the Operas of Wagner many times. His attachment to western music was both intellectual and emotional.

Often he read out to us pieces from poetry and prose which he liked. His

readings from T. S. Elliot were delightful. When he read passages from Virginia Woolf in his sing song voice you felt you even understood this rather difficult author. But I think it was Shakespeare that he loved best. He must have read every play of Shakespeare many times. He could lecture on any play better than any Oxford don – especially Hamlet and Tempest. I think Shakespeare gave a sort of direction to his life. He often told me that reading Tempest helped him in the attitude he had in doing research. I can easily imagine Chandrasekhar to be Prospero of the Tempest. If I now allow my imagination to run riot: Like Prospero he was –

Far away in a distant land – nursing a grievance and – biding his time for vengeance.

Then he was –

Drawn deep into books – succumbing to his overwhelming thirst for knowledge – and in splendid isolation – taking long years of preparation – and long years to perfect his art.

He discovered to his amazement –

The more he pursued his science (Shakespeare calls it the enchanted arts), the more single minded he became in its pursuit. Thus finally it cleansed him as it did Prospero.

When the time did come, a transformation had already taken place and like Prospero he had become free. His grievance was gone and he had no desire for vengeance.

However, I think what finally liberated him and his soul was his complete surrender to his god – whom he called 'The unmatched and unmatchable Newton'.

It would be impossible for me to convey to you the thrill and excitement of the exposition he gave us on Newton's *Principia* on one of our visits to Chicago. Almost true to his chosen title, *Newton's Principia for the Common Reader*, he lectured to Kausalya the Common Woman. I felt that he was really constructing the sentences that he was to write in the introduction two years later. We cherish a photograph of Kausalya and Chandrasekhar which I took. He is holding Cajoris 2nd edition of the *Principia* which he had bought for Eight Thousand Dollars.

Once when we were in his drawing room Kausalya asked 'Is that a Monet?',

'Yes – only a print – I cannot afford an original'. He told us that a chance remark by Roger Penrose that his excursions into General Relativity reminded him of the series paintings of Monet – triggered him off. It is the same Monet – whose Sunrise – an impression started the impressionist movement. He studied everything written about Monet and by Monet. He collected excellent prints of all his paintings – especially the Series Paintings of Haystacks, Poplars and Mornings on the river Seine. His word pictures of these paintings were such that he could make us see things we would not have seen otherwise.

I feel I am exploring the landscape of the mathematics of General Relativity as Monet explored his Haystacks which took new forms in the changing colour of light during the day and during different seasons.

He showed us how in Monet's poplars and their reflections were vertical lines while the tops of the other trees appeared near horizontal lines forming a wavy and beautiful grid – such exquisite wavy grids, according to him, inspired newer trends in geometric painting like those of Mondrian.

'The parallel in physics is', he said, 'just as the quantum theory of scattering of gravitational waves by stars led to a deeper understanding of the theories of scattering themselves'.

We sat there with our mouths open. It was an entrancing half hour. We can remember almost every word he spoke. Did we understand whatever he said. We do not know. His enthusiasm, his sparkling eyes, the cadence of his voice remained with us for a long long time.

Basilis Xanthopoulos

My visits to USA were never for more than 10 to 12 days. A day in Chicago was a must. I phoned him once. He said, 'I am flying over to meet Roger Penrose this evening. Can you come after three days?'. I went on the specified date. He received me at O'Hare airport. I again felt greatly privileged.

He was at that time probably working on black holes and cosmic strings.

Whenever I come across a stumbling block I go and meet Roger Penrose. We spend an hour together early in the morning when I present him with my problem. We have 4 to 5 hours of

discussion after lunch, then dinner, when we talk of other things – and I fly back. In no case has he not cleared up my doubts in physics or mathematics. An amazing man.

Chandrasekhar always followed where his mathematical and aesthetic instincts led him. During this period he collaborated with Basilis Xanthopoulos in Crete and Valeria Ferrari in Rome. He visited them often – but never for more than four or five days. It was with them that he discovered the underlying similarity between the mathematical theory of black holes and of colliding gravitational waves. We at the Raman Research Institute had the privilege of listening to his latest work which had not yet appeared in print. On every occasion during his formal or informal lectures, he mentioned with gratitude and affection Basilis Xanthopoulos and Valeria Ferrari.

So when I read the horrible story that while lecturing at his University, in Crete, Xanthopoulos was murdered by terrorists, I wrote to Chandrasekhar a condolence letter. He was touched and he wrote: 'My association with Basilis was the most enduring personal relationship I have had in all my 60 years in Science'. He also sent the foreword Basilis had written to Volume 6 of his collected works; This was published in *Current Science*.

When we met him next time and I referred to Xanthopoulos' death, we could see in his eyes the agony that I saw in my father's when he lost his much loved son. I realized then more than ever that those like Basilis Xanthopoulos and Valeria Ferrari were truly his Children – Children that he and Lalitha never had.

Return to India?

The following episode extended over four or five meetings in Bangalore, Chicago and Cambridge. I will try to give a connected account.

One day I asked him – you say in an essay you wrote about 30 years ago:

I have been a foreigner all these years except for a brief vacation for eight weeks when I was in India. By foreigner I mean that I have never been allowed to feel otherwise. Yet people from other countries migrate to distant

lands, build homes on new soils, adopt and are adopted by their neighbours. But why is it not so for me, an Indian.

I then asked 'has that feeling changed now?'

'Unfortunately no, I thought it would when we become citizens of USA in 1953. But even today I feel at home only in India. I think it is a great pity – why do you ask?'

I always poke my nose into other people's business. If you feel I offend you, stop me. Have you ever thought what will happen to you if Lalitha dies – and what will happen to Lalitha if you die. For I feel the US can become a very very lonely place.

(I remember he and Lalitha most graciously visiting the great physical chemist Mulliken. They took me with them. Mulliken was suffering from Alzheimer's disease. There was literally no one with him and Lalitha had to make tea for all of us.)

'Have you a solution?'

'Yes, I have.' Why don't you come back to India. Bangalore would probably be the best place. You can be at the Raman Institute. People always say RRI means Raman's Relatives Institute. He said 'some of his relatives who were at RRI have been very able scientists – of world renown'. I said, 'please do not interrupt me at this stage' and I continued. 'It would be nice if his most distinguished Relative can also be at RRI. You can stay with your brother who is in Bangalore. But it may be better for you and Lalitha to be in a cottage maintained by RRI or its Trust. It will provide you with help, transport, etc. You can invite any of your collaborators to Bangalore. Now and then, if you so desire, you can give courses of lectures. I can assure you very bright young people, and they are in plenty in India, will swarm around you. You can select the best of them to work with you. You would also be fulfilling Radhakrishnan's dream of having a Gravitation and General Relativity Group'.

He said 'I have also been thinking about this problem', and always the gentleman, he said 'It is most gracious of you to have thought of me and to have invited me. The offer is very at-

tractive. I shall surely think about it'.

I informed Radhakrishnan that there were chances – even though slim – of his accepting our offer. So a part of the new building was redesigned – special permission was obtained to put a lift – for Chandrasekhar had had his share of heart attacks and heart surgery.

He told me later he had given some careful thought – 'The only other place I could have considered was Oxford, because Roger Penrose is there. But there are some difficult conditions. Firstly, *I would not like to give up my US citizenship – for it was the US that gave me conditions that made my work possible, conditions which I could not have got anywhere else.* Secondly, I am not a rich man. I would get a pension. We need money to travel to conferences, to meet my colleagues and my collaborators. I am told that the Indian tax laws are formidable'.

I knew nothing about tax laws. I consulted the best people – the private and Government experts, I met persons at the highest levels. I was assured that Chandrasekhar could retain his US citizenship, even if he lived in India. They also assured me that steps could be taken to see that he was exempted from paying tax during his lifetime. Even an annual grant could be given to RRI for the sole purpose for the maintenance of Lalitha and he and for his research, travel, etc. I conveyed all this to him. At that time he had finished his work on Black Holes and had started his work on the collision of gravitational waves.

In 1983 he was awarded the Nobel Prize. I wrote him a congratulatory letter – also saying that my children were ecstatic, RRI and indeed the whole country were thrilled about it. A month later in January 1984 he phoned me saying that he would be accepting my invitation to give the inaugural address at the Golden Jubilee of the Indian Academy of Sciences – and that he was preparing an essay specially for the occasion. I was greatly excited.

He also conveyed the sad news that after getting the Nobel Prize it would be ungracious and appear ungrateful if he should leave the United States. Again a perfect Gentleman! He went on to thank me for all I had done and apologized that all my efforts came to naught.

Conclusion

In conclusion I say:

'There were two faces of Chandrasekhar, the stern aloof, and difficult to approach person and the kindly charming laughing human being dispensing jokes and anecdotes.'

'He had an inexhaustible supply of anecdotes – from his own association with scientists and from the history of science. He never repeated the same story to the same individual – For he had a phenomenal memory.'

'Each time you talked to him he opened up a new door for you and you saw a new vista – a new surprise'.

I was always flattered that he listened so intensely to one such as me. When I look back I shudder to think that I lived when he lived, that I knew him and he befriended me. For me this was the proof of the theory of Karma. I must have done something good long long ago.

About his science he said once 'I work on my own for my personal satisfaction generally outside the scientific mainstream'. It always became the mainstream a few years later. His earliest work led to the concept of the neutron stars and black holes. His survey of Brownian motion started new fields, many not at all related to astronomy. His concept of Dynamical Friction has become as much a part of the astrophysics vocabulary as the Chandrasekhar limit. With his work on Radiative Transfer began a brand new field in mathematics called invariant embedding. His ellipsoidal figures of equilibrium have become important to fast-rotating pulsars. One can go on and on.

His prescience in taking up any problem makes one gasp.

Believers would say – 'he was touched by the hand of God – a touch that remained with him till he died'. This of course would have made him very uncomfortable and even annoyed.

But there are simpler explanations. For him, like Srinivasa Ramanujan, doing science was like breathing. He did what he could not help doing – As the poet said:

'As in yonder valley the myrtle breathes its fragrance into space.'

MEETINGS/SYMPOSIA/SEMINARS

X Southern Regional Conference on Microbial Inoculants

Date: 10–11 December 1996
Place: Poondi

Sessions include: 1. Field applications of bacterial, algal and mycorrhizal inoculants, 2. Production problems, 3. Marketing problems, 4. Farmer's views on inoculants. Satellite meetings will also be conducted on biocontrol of pests and diseases, Training programme on sugarcane specific microbe *Acetobacter* species for sugar industries, Training cum awareness programme on biofertilizers for PG and research students on 12, 13 and 14 December 1996, respectively.

Contact: Dr C. Lakshminarasimhan
Convenor and Professor and Head
Department of Botany
A. V. V. M. Sri Pushpam College
(Bharathidasan University)
Poondi 613 503
Phone: 04374–39523

Mathematical and Theoretical Models in Biology

Date: 12–19 January 1997
Place: Puri

Applications are invited for participation in a workshop-cum-discussion meeting sponsored by the Department of Science and Technology to be held at Puri from 12 to 19 January 1997. The aim of the workshop is to expose advanced post-graduate students and young research workers to models in a whole range of areas in biology including immunology, developmental biology, genetics and behaviour. Participants are expected to be familiar with mathematical techniques at about the P. U. C. (12th standard) level.

Selected participants will have their travel (II class return rail fare) and living expenses at the venue of the workshop covered. A brief c. v. and statement of reasons for wanting to attend (within half a page) should be included. The last date for receipt of applications is 15 November 1996. Selected participants will be informed by the second week of December. Those interested should write to:

Dr V. Nanjundiah
Developmental Biology and Genetics Laboratory
Indian Institute of Science
Bangalore 560 012
Fax: 91–080–3341683

National Workshop on Remote Sensing and GIS Applications for Watershed Management

Date: 21–22 January 1997
Place: Bhopal

Themes include: 1. Applications of Remote Sensing and GIS in – a. Geology and Geomorphology, b. Landuse and Urban planning, c. Forestry, d. Soil and Agriculture, e. Environment, f. Water

resources. 2. Application of Remote Sensing and GIS techniques as a tool in watershed management.

Contact: Dr N. K. Tiwari
Secretary
ISRS, Bhopal Chapter
Remote Sensing Application Centre
M. P. Council of Science and Technology
26, Kisan Bhavan, Jail road, Arera Hills
Bhopal 462 004
Phone: 553224, 554578
Fax: 0755–553929, 554365
Telex: 0705–7394
E-mail: root%mpcost@sirnetd.ernet.in

National Symposium on GIS and Geological Remote Sensing

Date: 5–7 February 1997
Place: Tiruchirapalli

Themes include: Applied remote sensing in – Spectral behaviour of rocks and minerals, Digital image processing in geosciences, Lithological mapping, Structure and tectonics, neotectonics and seismotectonics, Geomorphology, Mineral and hydrocarbon exploration, Water resources, Engineering and environmental geomorphology, Geohazard zonation mapping and Geographical Information System.

Contact: Prof. SM. Ramasamy
Convenor
National Symposium on GIS and Geological Remote Sensing (NAGRES 1997)
Centre for Remote Sensing
School of Earth Sciences
Bharathidasan University
Tiruchirapalli 620 023
Phone: 420667
Fax: 0431–660245

National Conference on Energy Crisis and Environment

Date: 7–8 March 1997
Place: Chennai

Topics include: Non-conventional energy, Environmental energetics, Environmental Bio-technology, Environmental engineering, Environmental chemistry, Environmental management, Environmental ethics.

Contact: Dr Francis P. Xavier, SJ
Convenor/Director
Loyola Institute of Frontier Energy (LIFE)
Loyola College
Chennai 600 034
Phone: 8276894 Ext. 332
Fax: 044–8231684

Symposium on Tropical Crop Research and Development, India – International

Date: 9–12 September 1997
Place: Pattambi

ISTCRAD (International Society for Tropical Crop Research and Development) is organizing this symposium. Critical analysis on research/developmental activities in all the tropical crops are included in different sessions. Concurrent sessions are arranged

for all the disciplines including basic and applied research in agriculture and allied subjects.

Contact: Dr N. K. Nayar
Associate Director of Research
RARS, Pattambi
Kerala 679 306
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e-mail: kauhqr@ren.nic.in

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CURRENT SCIENCE

A fortnightly journal of research

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1. Mukundan, T. and Kishore, K., *Curr. Sci.*, 1991, **60**, 355-362.
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Acknowledgements should be brief. Footnotes are not allowed except to identify the corresponding author if not the first.

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COVER. Lifescape of the earth: In this depiction relative sizes of organisms are proportional to the number of species in the different groups known to science. These include: Monera: 4800; Fungi: 69,000; Algae: 26,900; Higher plants: 248,000; Protozoa: 30,800; Porifera: 5000; Cnidaria and Ctenophora: 9000; Platyhelminthes: 12,200; Nematoda: 12,000; Annelida: 12,000; Mollusca: 50,000; Echinodermata: 6100; Insecta < 751,000; Non-insectan arthropods: 123,400; Fishes and lower chordates: 18,800; Amphibians: 4200; Reptiles: 6300; Birds: 9000; Mammals: 4000. With 2.2% of the landscape and 0.6% of the seascape, India harbours 7.5% of the known diversity of the earth's lifescape. [Watercolour by Sanjeeva Nayaka.] See page 688.

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In this issue

Sálim Ali and the project 'Lifescape'

A special section is included in this issue of *Current Science* to mark the occasion of the birth centenary of the late Dr Sálim Ali – ornithologist extraordinary, naturalist par excellence, a great popularizer of natural history, a most inspiring teacher.

The project 'Lifescape', launched by the Indian Academy of Sciences to celebrate the birth centenary of Dr Sálim Ali, has been described by Madhav Gadgil on page 688. To begin with, this project plans to publish illustrated accounts of 2500–5000 species of living organisms – from the lowly viruses, bacteria, algae, fungi, through mites, insects, spiders and ferns, up to reptiles, birds, mammals and trees – found in the different parts of India. This exercise, even by itself, would be a fitting tribute to the memory of Dr Sálim Ali, who had remarked that 'the absence of illustrated books was, in my opinion, the most serious obstacle to the development of bird-watching'. Though Dr Sálim Ali completely demolished this obstacle by writing a series of outstanding books as far as birds are concerned, the drawbacks continue to be serious for other, less glamorous creatures. Now, though some are certainly more equal, all living organisms are equal – especially in the light of the Rio summit and the Biodiversity Convention. Preparation of such illustrated accounts of different groups of living organisms is in fact a fundamental necessity for fulfilling our national mandate of inventorying and monitoring biodiversity. (Of course, this could, in the process, lead to the discovery of the locations of rare, endangered but commercially highly lu-

crative species – and thus possibly abet the violation of the third mandate of conserving biodiversity; but it is an acceptable risk.)

However, these illustrated accounts are also meant to be the thin end of the wedge, aimed at enhancing the quality of science education in the country, to begin with, by suitable alterations in the syllabi. It is well-known that the study of biology, from the school level right up to the post-graduate level, places great emphasis on the text-book/laboratory approach – to the almost exclusion of the living world around us (though there are welcome signs of change, especially with the rising importance of environmental science). Madhav Gadgil points out that studying the living should be at least as important as studying the dead (i.e. watching birds, counting butterflies and collecting seeds should be as important as dissecting frogs). Students would no doubt wholeheartedly embrace this point of view! In his autobiography *The Fall of a Sparrow*, Dr Sálim Ali describes how, as a schoolboy, 'watching the behaviour and actions of his pet birds for hours together gave immense joy ...' and 'found this to be a far more pleasant way of passing time than doing homework'. And the new syllabus proposed in the article makes sure that not just homework, but even classwork involves activities of this kind. (It is tempting to speculate if students are opposed to classwork/homework per se, regardless of the intrinsic merit or interest of the topic. Assuming that the new syllabus is implemented, would the students a few generations from now equally wholeheartedly welcome a change from this crazy pursuit of creepy-crawlies in slushy swamps in pouring rain to the air-conditioned

comforts of dissecting a frog on the computer screen, using the latest virtual-reality software obtained by surfing on the internet.)

But, coming back to the Lifescape project, curriculum development is just one of its objectives. The more important goal is the compilation of the invaluable information generated from such studies involving projects by school, college and university students. For a nation as vast and as richly diverse as India, it is impossible either for the government or for any other central agency to collect country-wide information on location, distribution, abundance, etc. even for species of great economic importance – pests, pollinators, disease vectors and the like. On the other hand, student projects bring in thousands (if not more) of highly enthusiastic and motivated volunteers, who for weeks together would cheerfully brave all hardships of working in inaccessible places at unearthly hours, and generate high-quality information, while educating themselves about many subdisciplines of biology in the process. The article also describes in depth the philosophical underpinnings, the national status and the logistic aspects of the project.

A tribute to Dr Sálim Ali by one of his close associates J. C. Daniels, reproduced on page 686 of this issue, brings out the continued reverence felt towards the 'Old Man' by his colleagues and students. Of particular relevance is the description of gravity and care exercised by Dr Sálim Ali in the handling of public funds, a trait extremely worthy of emulation. Daniels also brings out the sense of wonder evoked by the enormous amount of valuable scientific information generated by Dr Sálim Ali,

with little more paraphernalia than a notebook, a pencil and a pair of binoculars (but then there was his extremely alert and analytical mind).

A brief sketch highlighting the quintessential features of Sálím Ali, the scientist, by Madhav Gadgil (page 685) describes Sálím Ali as 'the greatest whole organism biologist of 20th Century India' – (Now the phrase 'whole-organism-biologist' is a curious one – making one wonder if it includes those who work on whole viruses, whole bacteria and the like, and conjures up images of 'whole-molecule chemists' and 'whole-membrane-biophysicists' scoffing

at the lesser mortals who work merely on the constituent parts). Beginning with the description of an important experiment conducted by Dr Sálím Ali when he was all of eleven years old, the article traces his scientific career from Burma to Berlin to Bombay Natural History Society. It is interesting that the Burma stint was triggered by the evils of mathematics (logarithms and higher algebra) being taught in the first year of college. Mathematics thus seems to have made singularly important (and alas, totally unrecognized) contributions to the study of the ecology of the birds of Burma. The write-up also emphasizes what a pleasurable

reading Dr Sálím Ali's books make.

Talking about the status of ornithology in India in the 1920–1930, Dr Sálím Ali had described it as the 'Cinderella of Indian Zoology'. As of 1996, this poor sister has been magically transformed into a princess (if not the queen). There can be no doubt that this magic spell was cast almost entirely by a single individual. In the words of R. E. Hawkins

William Shakespeare's a master of words
And a tusker a leader of herds
But wherever you fare
Over land, sea or air
Sálím Ali is the raja of birds.

N. V. Joshi

Current Science

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CURRENT SCIENCE

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CORRESPONDENCE

Gene therapy

The introductory puff piece on page 339 and the article itself (P. N. Rangarajan and G. Padmanaban, *Curr. Sci.*, 1996, 71, 360-368) on Gene Therapy seemed strangely anachronistic in 1996. The authors seem to be totally unaware that gene therapy like King Balshazzar has been weighed in the balance of actual practice and found wanting. The US National Institutes of Health, having funded this narrow field for a decade at about \$200 million per year conducted its own in-house evaluation. This 'Report and Recommendations of the Panel to Assess the NIH Investment in Research on Gene Therapy (December 7, 1995)' finds that in spite of the huge expenditures *not one human has been cured* by gene therapy (see page 658).

Quite a record. The cost to society per life saved so far is infinite. Even after your authors' 'light at the end of tunnel' appears, those costs will not come down much. Indeed such high-tech medicine aimed at a few patients, however heart-rending the stories, is a major cost driver in medicine today. It is also a major attention-diverter for the scientific community away from the enormous problems in preventive medicine which affect tens of thousands.

Today research funds are driven more by the number of researchers and their need to be kept busy in their subspecialties. That is a major part of the cause of the impending crash of the US health care system. As a science policy analyst I have been emphasizing to governments that the *only* way to contain runaway research costs is to allocate research funds by the potential benefits, in this case the number of persons affected, and the seriousness of the disability. Those of us paid from the public purse for our research

must very seriously ask whether the public has a reasonable chance of obtaining any return on its investment. Or have scientists become 'welfare queens in white coats' expecting to be supported for doing our research irrespective of the outcome.

RUSTUM ROY

*Intercollege Materials Research
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Rangarajan and Padmanaban reply:

It is easy to be critical and cynical with information interpreted to one's own way of thinking. Every emerging area including molecular biology as a field and recombinant DNA as a technology have faced strident criticisms in terms of utility, before they were accepted as powerful tools not only in basic research but also in terms of applications. When the introduction of a single new drug into the market is estimated to take 15 years and in excess of \$300 million, we are surprised that gene therapy is expected to deliver on its promises within a decade of investment. The euphoria created on gene therapy due to public pressure, commercial interests and media hype is well known. We are quite aware of the NIH panel report, which has characterized NIH spending of \$200 million as 'appropriate', but has recommended instituting short-term grants to fund innovative research and to test new ideas (*Nature Medicine*,

1996, 2, 7-8). Another commentary states 'Human gene therapy has not yet come of age, but there can be no justification for doubting its eventual success, as an adjunct to traditional therapies or as a definitive therapy on its own. The most revolutionary aspect of human gene therapy has been the conceptual one and that phase is over. We have now reached the difficult evolutionary stage of making it work.' 'Human gene therapy is not yet ready for prime time at least at the clinical level. But it is ready for and needs prime time nurturing and development' (*Nature Medicine*, 1996, 2, 144-147). Hopefully, these will not be considered as anachronistic in 1996! The consensus is to 'go back to the basics' and fill in the missing links as discussed in our article. The fact remains that gene therapy offers hope of cure not only to genetic disorders, but also to cancers and infectious diseases. Infact, serendipity has led to the birth of DNA (genetic) vaccinations, a facet of gene therapy research that holds tremendous promise. One is not just thinking of unaffordable treatment to a few patients, but approaches based on direct DNA injection, genetic immunization, etc., that will be cost effective and benefit millions, especially in the developing world. Once again, there are hurdles to cross and these are worth crossing.

Professional science analysts and those who are in a position to advice governments should necessarily have a balanced view. It is easy to give populist advice such as to allocate research funds on the basis of potential benefits, number of persons affected, seriousness of disease, etc. Such advice will be lapped up by politicians but history is replete with instances of blue sky research, serendipity

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as well as goal-oriented research contributing to human welfare. While, governments in general should be helped to decide on priorities, gene therapy as such has tremendous possible applications for mankind and sweeping gener-

alities will only harm a great cause. Let us not straight jacket issues and make scientists appear as in 'white coats for self-preservation'. The community is as much interested in human welfare as any one else professing the same.

P. N. RANGARAJAN
G. PADMANABAN

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NEWS

Report and recommendations of the panel to assess the NIH investment in research on gene therapy

Executive summary of findings and recommendations

Dr Harold Varmus, Director, National Institutes of Health (NIH), appointed an *ad hoc* committee* to assess the current status and promise of gene therapy and provide recommendations regarding future NIH-sponsored research in this area. The Panel was asked specifically to comment on how funds and efforts should be distributed among various research areas and what funding mechanisms would be most effective in meeting research goals.

The Panel finds that:

1. Somatic gene therapy is a logical and natural progression in the application of fundamental biomedical science to medicine and offers extraordinary potential, in the long-term, for the management and correction of human disease, including inherited and acquired disorders, cancer, and AIDS. The concept that gene transfer might be used to treat disease is founded on the remarkable advances of the past two decades in recombinant DNA technology. The types of diseases under consideration for gene therapy are diverse; hence, many different treatment strategies are being investigated, each with its own set of scientific and clinical challenges.
2. While the expectations and the promise of gene therapy are great, clinical efficacy has not been definitively demonstrated at this time in any gene

therapy protocol, despite anecdotal claims of successful therapy and the initiation of more than 100 Recombinant DNA Advisory Committee (RAC)-approved protocols.

3. Significant problems remain in all basic aspects of gene therapy. Major difficulties at the basic level include shortcomings in all current gene transfer vectors and an inadequate understanding of the biological interaction of these vectors with the host.
4. In the enthusiasm to proceed to clinical trials, basic studies of disease pathophysiology, which are likely to be critical to the eventual success of gene therapy, have not been given adequate attention. Such studies can lead to better definition of the important target cell(s) and to more effective design of the therapeutic approach. They often can be carried out in appropriate animal models. Pathophysiologic studies may also suggest alternative treatment strategies.
5. There is a clear and legitimate need for clinical studies to evaluate various aspects of gene therapy approaches. Although animal investigations are often valuable, it is not always possible to extrapolate directly from animal experiments to human studies. Indeed, in some cases, such as cystic fibrosis, cancer, and AIDS, animal models do not satisfactorily mimic the major manifestations of the corresponding human disease. Clinical studies represent not only practical implementation of basic discoveries, but also critical experiments which refine and define new questions to be addressed by non-clinical investigation.
6. Interpretation of the results of many gene therapy protocols has been hindered by a very low frequency of gene transfer, reliance on qualitative rather than quantitative assessments of gene transfer and expression, lack of suitable controls, and lack of rigorously defined biochemical or disease endpoints. The impression of the Panel is that only a minority of clinical studies, illustrated by some gene marking experiments, have been designed to yield useful basic information.
7. Overselling of the results of laboratory and clinical studies by investigators and their sponsors—be they academic, federal, or industrial—has led to the mistaken and widespread perception that gene therapy is further developed and more successful than it actually is. Such inaccurate portrayals threaten confidence in the integrity of the field and may ultimately hinder progress toward successful application of gene therapy to human disease.

Based on these findings, the Panel recommends the following:

1. In order to confront the major outstanding obstacles to successful somatic gene therapy, greater focus on basic aspects of gene transfer, and gene expression within the context of gene transfer approaches, is required. Such efforts need to be applied to improving vectors for gene delivery, enhancing and maintaining high level expression of genes transferred to somatic cells, achieving tissue-specific and regulated expression of transferred genes, and directing gene transfer to

*Stuart H. Orkin and Arno G. Motulsky, Co-chairs.

- specific cell types. To stimulate innovative research, the Panel recommends the use of interdisciplinary workshops, specific programme announcements in these areas, and the use of short-term, pilot grants for testing new ideas and for encouraging investigators from other areas to enter the field of gene therapy.
2. To address important biological questions and provide a basis for the discovery of alternative treatment modalities, the Panel recommends increased emphasis on research dealing with the mechanisms of disease pathogenesis, further development of animal models of disease, enhanced use of pre-clinical gene therapy approaches in these models, and greater study of stem cell biology in diverse organ systems.
 3. Strict adherence to high standards for excellence in clinical protocols must be demanded of investigators. Gene therapy protocols need to meet the same high standards required for all forms of translational (or clinical) research, whatever the enthusiasm for this (or any other) treatment approach.
 4. To enhance the overall level of research in this area, the Panel recommends that NIH support broad interdisciplinary post-doctoral training of M.D. and Ph.D. investigators at the interface of clinical

- and basic science. Mechanisms for physician training in this area might include use of career development awards based on a programme announcement in gene therapy.
5. Investigators in the field and their supporters need to be more restrained in their public discussion of findings, publications, and immediate prospects for the successful implementation of gene therapy approaches. The Panel recommends a concerted effort on the part of scientists, clinicians, science writers, research advocates, research institutions, industry, and the press to inform the public about not only the extraordinary promise of gene therapy, but also its current limitations.
 6. NIH has already provided an appropriate initial investment in gene therapy. Future gene therapy research should compete with other forms of biomedical research for funding under stringent peer review. Only with fair, yet critical, peer review will high standards be met and maintained. The Panel specifically does not recommend special gene therapy study sections, expansion of existing centre programmes in gene therapy, or expansion of the recently funded core vector production programme. To ensure that the level of support remains appropriate, the

NIH investment in this field should be reexamined periodically.

7. To enhance the contribution of industry to the field, the Panel recommends that NIH encourage collaborative arrangements between academic institutions and industry that complement NIH-supported research, and also implement mechanisms that facilitate the distribution and testing of vectors and adjunct materials for use in clinical studies.
8. In an effort to improve gene therapy research and reduce duplication of effort, the Panel urges better coordination and scientific review of such research throughout the NIH Intramural Programme. In addition, NIH Institute Directors should resist pressures to include gene therapy research in their portfolios (either intramural or extramural) to 'round out' their programmes or compete with other Institutes. Instead, they should include such research only when there are compelling scientific reasons to go forward. Institute Directors should take the lead, where it seems appropriate, to focus efforts on improvement of diagnosis and understanding of disease pathogenesis and await further developments in vector technology before expanding clinical gene therapy programmes.

Asteroids and Earth's evolution – A new perception

Shaking the very foundations of long-accepted geological and geophysical theories, some new unorthodox views which cannot be brushed aside have been put forward by H. R. Shaw, a researcher in the US Geological Survey, in his recent book *Craters, Cosmos and Chronicles: A New Theory of Earth* (Stanford University Press). Shaw feels that asteroids and comets had a much greater role in shaping Earth's geological evolution than has been presumed so far. These celestial bodies did not impact randomly all over Earth, but hit only particular spots, determined by their nonlinear interactions with members of the solar system. The repeated batterings that the Earth has taken in its long past have contributed to the various geophysical phenomena

like the distribution of continents, disposition of Earth's magnetic field, triggering of volcanic eruptions and the evolution and extinction of life forms.

Along with colleague William Glen, he chronicled several asteroid impacts between the time period 50–100 million years ago, and surprisingly found that their impact sites fell along a line encircling the globe, which they aptly called the K–T swath, after the famous asteroid impact during the Cretaceous–Tertiary period (Figure 1). Still older impact sites dating back to 600 million years ago were also found to group into three distinct clusters in North America, Eurasia and Australia, instead of being randomly distributed among all continents.

Shaw feels that chaos arising from

nonlinear interplanetary gravitational influences dictates the orbits assumed by asteroids which are believed to have been ejected out of the main asteroid belt. Their chaotic trajectories bring them into inner solar system, where they come within the grip of the Earth (or other inner planets) and once within its influence, the uneven distribution of mass inside the Earth causes the paths of these asteroids to shift gradually to specific orbits, in tune with the fluctuating gravity profile over the Earth. In due course, they end up crashing to the Earth, always along circular tracks corresponding to their gravity-tuned trajectories. According to Shaw, these bodies have been crashing along a limited array of sites or 'cratering nodes' over the past half-billion years.

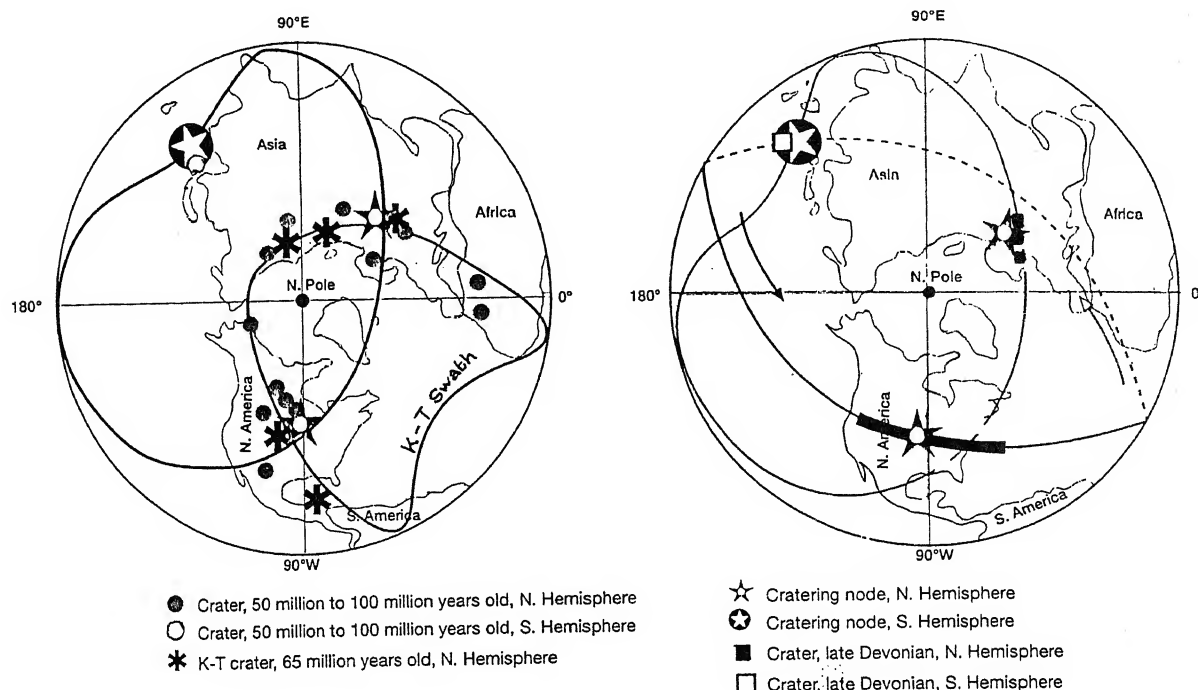


Figure 1.

After elaborate computer calculations, Shaw has derived a potential orbit for Earth-circling asteroids.

While the postulated mechanics of asteroid capture appear plausible, their role as prime agent shaping Earth opposes well-accepted theories. For example, Shaw feels that their powerful impacts in the past triggered some of the flood basalt eruptions which he finds located along the great circle. This runs counter to the accepted idea that they arise as plumes of hot lava from Earth's molten interior. Similarly, his hypothesis is

opposed to current theories of plate tectonics, or the drifting of continents. He finds that the regions frequently hit, i.e. the cratering nodes, have remained the same during the vast span of 600 million years, thereby implying that the continents have not drifted for long periods, or they did so, much less than what is believed, or they have been returning again and again to the same location. Even the well-documented shifting of the Earth's magnetic field, believed to be caused by drifting currents of molten iron within the Earth's core is attributed by Shaw to

powerful asteroid impacts inducing the drifts.

Although a few scientists in the past have linked asteroid impacts to the onset of volcanism and drifting of magnetic field, it is however Shaw who unified these by applying nonlinear dynamics and predicting patterns in their paths, otherwise not apparent.

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RESEARCH NEWS

The manganese hide out in the photosynthetic reaction centre

M. K. Raval

A manganese cluster oxidizes water to oxygen in the reaction centre II of oxygen evolving photosynthetic organism. This photochemical reaction is crucial for production of biomass and maintenance of

oxygen level on earth. Therefore, the study of structural and functional aspects of the Mn-centre has received much attention¹. Knowledge of structural organization of Mn and its ligands in the

catalytic centre and mechanism of photo-oxidation of water might lead to design of synthetic Mn-centre mimicking photosynthetic reaction centre II for harvesting solar energy². However, it has not been

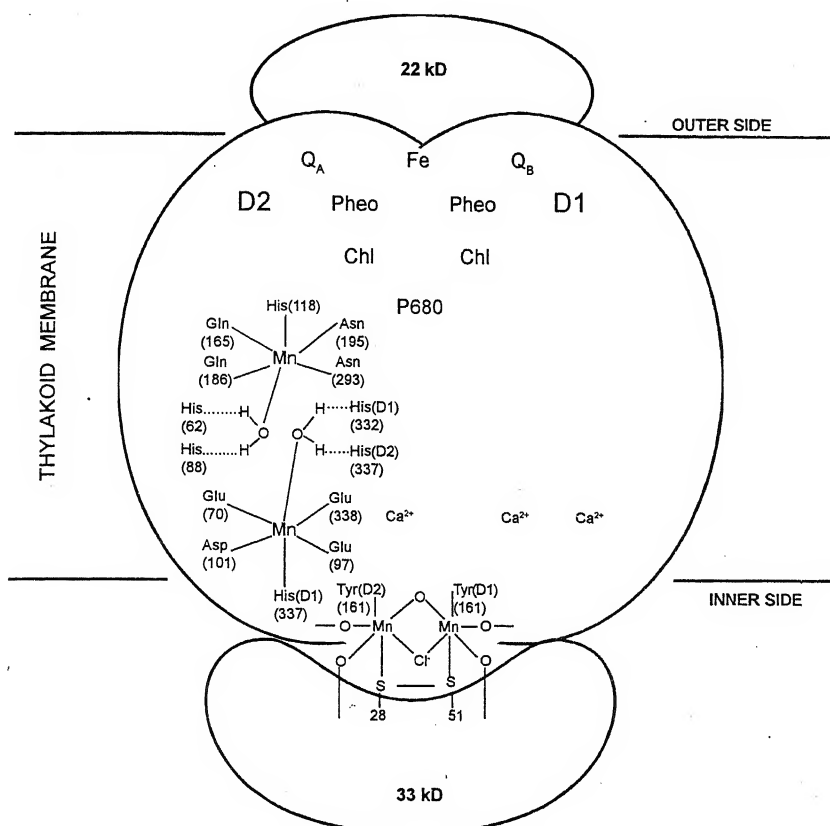


Figure 1. A model for structural organization of Mn in photosynthetic reaction centre II of higher plants⁹. D1, D2: heterodimeric proteins of reaction centre II; P680: Special pair Chlorophyll a absorbing at 680 nm; Chl: Chlorophyll a; Pheo: Pheophytin a; Q,A: Quinones A and B.

possible to discover the nature of this cluster (two dimers or a tetramer) as well as the hide out (amino acid residues ligating to Mn cluster) in the heterodimer proteins D1/D2, even after an untiring endeavour of almost two decades by scientists with elegant techniques like electron spin resonance (ESR)³ and extended X-ray absorption fine structure spectroscopy (EXAFS)⁴. X-ray crystal-

lographic structure determination of purple bacterial photosynthetic reaction centre and its similarity with oxygen evolving reaction centre II, inspired building of a number of models for the catalytic site of reaction centre II.

Recently, site-directed mutagenesis has shown promise to locate the manganese hide out. Debus and colleagues have reported some amino acid residues as

probable ligands to Mn through mutant study in cyanobacteria^{5,6}. The probable Mn binding ligands are H332, E333 and H337 of D1 protein. Earlier in 1990 Vermaas *et al.*⁷ had reported probability of E69 of D2 protein in cyanobacteria (E70 of D2 in higher plants)⁷. These results coincide with theoretical propositions made earlier. We had reported in 1990 in a theoretical model of reaction centre II, H332, H337 of D1 and E70 of D2 as ligands to Mn (Figure 1)⁸. The theoretical model of Coleman and Govindjee also proposes E70 of D2 and E333 of D1 to be possible Mn ligands⁹.

Though cumbersome the site-directed mutagenesis appears to be the only effective technique next to X-ray crystallography to discover the Mn hide out.

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Research Snippets (edited by K. Manjula)

Giant magnetoresistance: New concepts

Magnetic coupling has played a key role in the discovery of the Giant Magnetoresistance (GMR) effect in thin films. In certain multilayers consisting of alternating ferro- and non-ferromagnetic films

(e.g. Co/Cu), adjacent ferromagnetic films couple to each other through the nonferromagnetic film separating them. When an external magnetic field is applied to overcome this coupling, the ferromagnetic moments align and as a result, a change in the electrical resistance of an unprecedented magnitude is observed. This is referred to as Giant Magnetoresistance or GMR effect.

The large drop in electrical resistance of a solid in a magnetic field is an important phenomenon that has a potential application in magnetic storage media like magneto-optical recording and magnetic sensing devices. Lanthanide manganites doped with alkaline earth metals like calcium or strontium having the formula $\text{La}_{1-x}\text{A}_x\text{MnO}_3$, (A = Ca, Sr) exhibit a

perovskite structure. Such oxides also exhibit a spectacular magnetoresistance effect and suppress the resistance in magnetic field of ~ 6 Tesla to $\sim 100\%$ at room temperature. While its utility is yet to be exploited commercially, at the fundamental level, the underlying cause of GMR effect has to be understood.

Among the hypotheses put forth so far by experts in this field, one hypothesis believes that the crucial cause of GMR is the so-called double-exchange (DE) phenomenon which involves the hopping of electrons between heterovalent $\text{Mn}^{3+}/\text{Mn}^{4+}$ pairs in the lanthanide oxide. Additionally, a strong Jahn-Teller distortion associated with the octahedrally coordinated $\text{Mn}^{3+} : 3d^4$ experienced in these materials also contributes to GMR.

An interesting development is the discovery of the GMR effect in another manganese oxide ($\text{Ti}_2\text{Mn}_2\text{O}_7$) which exhibits an entirely different type structure (the 'pyrochlore structure'). A recent paper in *Science* by Subramanian *et al.* (1996, 273, 81) has established that the GMR effect caused by this oxide is due to neither of the two earlier effects noticed among the earlier oxides. This new oxide is actually a stoichiometric compound having no mixed valency that is characteristic of the $\text{Mn}^{3+}/\text{Mn}^{4+}$ group. It has been postulated that magnetic ordering driven by superexchange and a strong spin-fluctuation scattering above the Curie temperature are the causes of GMR in this oxide. In addition, thallium(III) has also been implicated to have a role in GMR, as it tends to complicate the electronic structure by its own mixed valency ($\text{Ti}^{\text{I}}/\text{Ti}^{\text{III}}$). This role has already been well-established in thallium cuprate superconductors.

J. Gopalakrishnan

Once upon a (Jurassic) time...

Was the Archaeopteryx really the 'early bird'? For that matter, during the course of evolution, did crocodiles ever go vegetarian? Recently, old skeletons that have tumbled out from the lands of the Far east have provided several interesting answers to these queries. Avian fossils from

the Liaoning province in Northern China have thrown light on the existence of a new fossil bird, about the size of a pigeon, that is reverently referred to as *Confuciusornis sanctus* (Lian-Hai Hou *et al.*, *Nature*, 1995, 377, 616). Its external features bear the resemblance of both the modern birds (in the Cretaceous times) as well as the bird that existed during the earlier period (viz. the Archaeopteryx). These discoveries are considered to be very exciting in as much as they have questioned the existing views of Archaeopteryx ancestry to modern birds. Evidence now points out to a possibility of the existence of other side branches to the Avian tree. While the Archaeopteryx may also be one such branch, it had somehow met with a dead end. These findings lead to the obvious conclusion that if birds were already diverse in the Jurassic period, the still more primitive of the species must have taken to wings as early as the late Triassic—some 60 million years before Archaeopteryx even flapped its own, in the Jurassic skies.

Three decades ago, Chinese geologists surveying for petroleum in the Hui province of Central China stumbled upon a fossil of a strange animal that probably existed 120 million years ago. The fossil that found a home in Toronto museum was recently re-examined and has been identified as belonging to the crocodile family (Xiao-Chun Wu *et al.*, *Nature*, 1995, 376, 678). The reptile, called *Chimeraesuchus paradoxus*, showed a variation in its skeletal features to other crocodiles. The most striking difference was that unlike the sharp conical teeth characteristic of carnivorous crocodiles, this reptile had multicuspid molar teeth which strongly indicated that the latter was actually herbivorous. This was obviously the period of the 'green (r)evolution'!

A. V. Sankaran

Small is beautiful

One of the objectives in the study of materials today is the synthesis and design of matter at a nanometer scale. Patterning material at this scale is helpful in the manufacture of miniature electronic components. In 1856, Michael Faraday had observed that material properties are

strongly size-dependent and thus can actually be tuned to sensitivity.

Earlier approaches towards the preparation of coupled quantum dots included the co-colloids of cadmium selenide-zinc oxide and cadmium sulphide-silver iodide. Aggregates of gold could also be synthesized using small molecule cross-linking agents. While the properties of nanocrystals have been studied earlier using organic monolayers and crystallization, it was not known whether self-assembly methods could be employed to generate complex nanocrystals.

Molecules in biological systems are held together several types of bonds including the strong covalent force as well as the weaker hydrogen bonds. Due to the complex nature of recognition systems that exist in biological organisms, it becomes necessary for such bonds to be specific and precise. Can biological molecules be exploited in the design of nanostructures?

Paul A. Alivisatos and his colleagues at the University of California at Berkeley, USA have addressed this question in an elegant manner (as reported in *Nature*, 1996, 382, 609–611). Small stretches of DNA (oligonucleotides) were modified at their ends with sulphhydryl groups. These were then coupled with an excess of mono-maleimido-gold particles. Following this coupling, the oligo-gold conjugates were then combined with set amounts of complementary single-stranded oligos. The resulting double-stranded complexes were then purified and analysed both with biochemical methods as well as through direct observation using transmission electron microscopy.

Results demonstrate that the use of oligonucleotides in the synthesis of nanoparticles has great potential. For one thing, by altering the base composition of the oligonucleotide, it is possible to generate flexibility in the oligo structure. Due to the nature of bonds that exist in DNA, it is possible to reverse reaction using heat, unlike the situation that exists when organic synthetic methods are used. With the further improvement in the methodology like the use of shorter and more rigid linkers and the use of other ligands apart from gold, a wider range of physical phenomena can be investigated in nanostructure architecture. For the present, one can but admire the 'gold zari' made out of Nature's own exquisitely beautiful threads of DNA.

Turmeric – the golden spice

The dictionary defines 'turmeric' as an aromatic rhizome of an East Indian zingiberaceous plant, *Curcuma longa*. Curcumin (diferuloylmethane) is the yellow pigment in turmeric used as a spice, food coloring and as a preservative. The powder of the plant is used often as a condiment as in curry powder or as a yellow dye or as a medicine. Over the recent months, this plant has received much attention due to the challenge of the patent filed by a university overseas for the discovery of its wound-healing properties. Organizations in India maintain that this property has been very well-established in India and is not a novel finding in itself. While the debate is proceeding at a leisurely pace one could look at the actual scientific facts on this herb that have been established over the years in the scientific literature. A medline search revealed many interesting reports. Here are few published reports on the biochemistry of turmeric.

Peroxidation of lipids in the living body is known to destroy the cell membrane and ultimately lead to the premature ageing of an individual. Last year, a report on the isolation of the turmeric anti-oxidant protein (TAP) was isolated from an aqueous extract of turmeric (Selvam *et al.*, *J. Ethnopharmacol.*, 1995). This

protein was shown to inhibit unwanted lipid peroxidation in the rat system.

In September 1995, *Cancer Letters* reported a study conducted at the Rutgers State University of New Jersey. During chronic inflammation, chemicals called cytokines are released in the human body that in turn induce the release of nitric oxide. This causes considerable damage to DNA and can even lead to carcinogenesis. This study concentrated on the effect of curcumin (the active principle of turmeric) on inflammation. It was found that curcumin inhibited the nitrite production in mouse peritoneal cells to an extent of 75%. Turmeric was implicated to be an anti-inflammatory as well as an anti-cancer causing agent.

Compounds like eicosanoids can cause the aggregation of platelets that ultimately leads to inflammation at the site of the wound. A study carried out in Denmark by Srivastava and his colleagues (*Prostaglandins Leukot Essent Fatty Acids*, April 1995) revealed that turmeric inhibits the platelet aggregation by modulating the eicosanoid biosynthesis. This could pave the way for the development of drugs that block key enzymes involved in the eicosanoid biosynthesis.

At the National Cancer Institute, Maryland, the effect of turmeric on HIV infection was studied by Mazumdar and

his colleagues (*Biochem. Pharmacol.*, April 1995). Clinical trials in AIDS patients have revealed that turmeric actually blocks the functioning of the HIV integrase enzyme (of HIV type 1) that is required for the pathogenesis of the disease. Inhibition of an integrase deletion mutant containing only amino acids 50–212 suggests that curcumin interacts with the integrase catalytic core. This data suggests that HIV integrase inhibition could contribute to the antiviral activity of curcumin, giving hope to the development of new strategies for antiviral drug development that could be based on curcumin.

Several other interesting findings have been catalogued in the databank available on the wonderful uses of this exquisite herb. In essence, it would do one well to garnish generously the rasams, sambars and all that comprises the spicy Indian cuisine with more than a dash of this golden spice.

The patents that have so far been filed for its uses are numerous over the last couple of years. The recent patent list from the databank records a variety of findings that range from the use of turmeric as a dye to its use in the detection of cyanide adulterated food products. However, that is an entirely different story altogether.

SCIENTIFIC CORRESPONDENCE

Comments on 'The end phase of sedimentation of Krol Belt succession in Nainital syncline – Stratigraphic analysis and fossil levels' (D. K. Bhatt, *Curr. Sci.*, 1996, 70, 772–774)

The propositions of the author that 'both in terms of lithofacies and chronostratigraphy, the presence of Tal sequence in Nainital and the reported Ediacaran fossils from Nainital do not fulfil basic palaeontological requirements to be acceptable', are strongly refuted as they are not based on basic observable facts in the field. The following discussions would justify our points.

Detailed lithostratigraphic correlation and basin analysis based on lithofacies isopach maps of Blaini–Krol–Tal

sequence between Solan and Nainital^{1–4} have clearly demonstrated that the Tal Formation pinches southeast to Rikhnikhali in the Garhwal syncline and does not continue in Nainital area. The arenaceous beds in the form of rhythmite as well as thin quartz arenite bands exposed in the core of the Nainital syncline start appearing even in the upper part of the Krol sequence in the southeastern closure of the Garhwal syncline. This rhythmite horizon, which yields Ediacaran fossils in Nainital^{5,6}, Garhwal, Mussoorie and

Nigalidhar synclines^{4,7} belongs to the Upper Krol Formation (Krol-D) and not to the Tal Formation^{8–10}, which is well exposed in the Garhwal, Mussoorie, Korgai and Nigalidhar synclines^{1–3} and yielded characteristic fossil assemblages of Meishucunian to Tsanglangpuian stages^{11–17} of early Cambrian age, overlies the Ediacaran fossil yielding Krol Formation^{4,7}. The Ediacaran fossiliferous horizon in the upper part of Krol Formation (Krol-D) is characterized by $\delta^{13}\text{C}$ values that vary from 1.3 to 1.5‰ PDB in the

SCIENTIFIC CORRESPONDENCE

Garhwal syncline^{18,19}. Similar isotopic signatures have been described from other Ediacaran fossiliferous horizons in the world²⁰.

The horizon from which Ediacaran fossils were recorded, occurs near the top of the Krol-D sequence exposed in the core part of the Nainital syncline⁴⁻⁶. The level from which so-called 'chert-phosphorite Member of Tal Formation fauna' comprising *Coleoloides typicalis* Walcott and *Olivoides multisulcatus* Qian recorded by Bhatt and Mathur¹⁰, is stratigraphically well below the level from which we recorded Ediacaran fossils^{5,6}. The law of superimposition of strata does not permit beds with younger fauna to be underlying beds with older fauna unless the sequence is inverted, which certainly not is the case in Nainital Syncline. It is, therefore, clear that the beds from which fauna is described by Bhatt and Mathur¹⁰, is well within the Krol Formation (Krol-D).

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D. K. Bhatt replies:

The disagreement of views pointed out by Shanker and Mathur relates not so much as to the observable facts in the field, but to the interpretation of the field observations, so far as the development of the Krol-Tal stratigraphy in the Nainital area is concerned. A host of recent workers on the basis of regional field observations¹ and/or detailed field work²⁻⁴ have demonstrated that not only the entire succession of the Krol Formation up to the level or Krol-E is present in the Nainital syncline, but also the additional strata described as Krol-F⁴ or Tal². This inference is supported by the recent record of small shelly fossils⁵.

The delineation of the extent of lithostratigraphic units within Proterozoic basins, having thousands of metre thick stratigraphic development and spanning

hundreds of million years, cannot be and should not be based solely upon 'lithofacies isopach maps'. So far as I am aware there exists no parallel example anywhere in the world where similar practice has been followed for determining the lithostratigraphic succession of a Proterozoic basin.

It may be reiterated that the topmost 75-80 m of the Krol Formation and the overlying 75 m thick Giwalikhet Member of the Tal Formation in the section at Nainital have yielded elements of small Shelly fossils⁵ that characterize strata of Meishucunian age elsewhere in the Krol Belt^{6,7}. The 'Ediacaran fossils' of Shanker and Mathur come from Narainagar Member of the Tal Formation that forms the youngest lithounit within Tal, overlying the Giwalikhet Member, in the Nainital section. Thus the 'Ediacaran fossil' level of Shanker and Mathur marking the strata of 'Ediacaran age' lies some 150 m above the horizon, revealing the oldest record of small shelly fossils of Meishucunian age (see paper under discussion and Figure 4 in ref. 5). This becomes chronostratigraphically incompatible, for the simple reason that the Ediacaran age has to precede the Meishucunian age according to the global chronostratigraphic scale.

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Association of transglutaminase-like antigen with cytoskeletal proteins

Almost simultaneously but independently two papers dealing with an unique association of transglutaminase (TGase) with that of intermediate filaments (IF) such as keratins¹ and vimentin², appeared. The evidence for the novel association of TGase with keratin (58 kDa) stems from biophysical and biochemical analyses of keratin filaments, which elicited a greater order of aggregation in the presence of divalent cation such as calcium. The addition of the chelator, i.e. EDTA abolished the calcium-mediated aggregation of the reconstituted keratin filaments (RKF). This implicates the exquisite specificity for calcium which functions as a cofactor for the enzyme. Addition of effectors such as cystine and histamine to the RKF resulted in a significant reduction in the TGase activity as well as kinetics of an order of aggregation. Further, Western blot obtained with polyclonal antibody against guinea pig liver TGase cross-reacts with the 58 kDa protein in the urea soluble fraction from rat vaginal epithelium. This suggests that the guinea pig liver tissue TGase and the 58 kDa keratin share a common epitope as indicated in the published work earlier¹.

In line with the above mentioned data, Trejo-Skalli *et al.*² mainly using immunobiological techniques, have demonstrated an association of TGase related antigen with another cytoskeleton protein widely present in the fibroblasts called vimentin. By using double label immunofluorescence antibodies to vimentin and GP2.1.2 (a monoclonal antibody raised against guinea pig liver TGase) the authors have demonstrated *in situ* that TGase-like antigen cross-react with vimentin-positive fibroblasts. After subculturing of the primary cultures, a proportional increase in the percentage of GP2.1.2 positive cells is evident. Colchicine treatment did not perturb this association, indicating that the association is very stable. Morphological observations are further supported by the findings of Western blot data where GP2.1.2 recognizes a 280 kDa protein complex of vimentin with TGase in the IF-enriched cytoskeletal preparation but does not cross-react only with vimentin per se. Further when this anti-

body was injected into the live dermal fibroblasts it eventually resulted in the collapse of the vimentin IF network.

What could be the physiological implication of association of TGase with cytoskeletal proteins? An analysis of the subcellular distribution of TGases may reveal clues regarding their mechanism of action. TGases encompass an array of biological functions and its diversity and specific expression in different types of cells are attributed to the physiological requirement of the cell. In recent years, there is an increasing interest in TGases because of their involvement in rendering post-translational modifications in a variety of tissues. Most notable is the cross-linking of proteins by Σ (τ -glutamyl)lysine which is a covalent cross-linkage and shows resistant to the treatment by any known enzyme so far and also by many denaturants^{3,4}.

Some of the other major functions of TGases include irreversible membrane stiffening of the erythrocyte, receptor-mediated endocytosis and regulation of cellular growth and its differentiation by tissue TGase⁵, formation of insoluble cornified envelopes in epidermal keratinocytes by epidermal TGase⁶, formation of vaginal plugs by prostrate TGase⁷, formation of insoluble fibrin clots catalysed by factor XIIIa⁸, and in wound healing the cross-linking of fibronectin to collagen is brought about by factor XIIIa, and nerve regenerating process observed in optic nerve by nerve TGase^{9,10} and apoptotic process by tissue TGase^{11,12}.

Several reports are available to demonstrate the association of IF with various kinases like calcium and cAMP independent and dependent kinases^{13,14}. Other cytoskeletal filamentous structures like microtubules and microfilaments also show an association with the glycolytic enzymes. This association permits localized enrichment of these enzymes for energy dependence and the cytoskeletal matrix interactions¹⁵. Earlier studies on HT29 or NC-N417 cells have demonstrated the association of a kinase such as PKC Σ and cytokeratin fractions¹⁶.

A functional role of these associated TGases has not yet been ascribed. We can hypothesize that depending upon the biological signal the cell receives the corresponding TGase which may be activated to elicit its transamidating function. A key observation in our data is the demonstration of the catalytic potential for the transamidating activity of the keratin fraction by its ability to incorporate labelled ¹⁴C-spermidine substrate. *In vitro* vimentin was found to serve as a substrate for TGase. In skin and in VEC the cross-linking of keratins results in the formation of N Σ (τ -glutamyl)lysine bridges which contribute to the stabilization of the filament network.

An indepth understanding of this association would not only unravel the molecular mechanism underlying the protein-protein interactions but also paves way in understanding the physiobiophysical significance of this association as their specific substrates are found among the constituent component of the IF network itself.

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Role of animals in the spread of human ringworm disease in Madras

Dermatophytosis in animals is an important public health problem, since this disease is frequently transmitted directly or indirectly from domestic and wild animals to man by contact^{1,2}. Several workers have reported zoonotic ringworm disease of man from different parts of India³⁻⁵. Here we report the isolation of three strains of *Trichophyton mentagrophytes* from animals and their epidemiologic importance in human ringworm disease in Madras since such data is not available.

One lion cub, one Nilgiri langur, three lamas, and three camels in Arignar Anna Zoological Garden and 22 dogs (17 native breeds, 2 German Shepherds, 2 Doberman, 1 pomeranian), 11 cattle and 1 donkey calf in and around the city of Madras were screened. The animals were thoroughly examined for the presence of any lesion suggestive of ringworm with the help of a veterinary officer. The fur was then combed with a sterile tooth brush and then stabbed onto Sabouraud's dextrose agar plates with and without antibiotics (chloramphenicol 0.5 mg/ml, cyclohexamide 0.5 mg/ml). The plates were incubated for 6 weeks at 26°C. Identification was done based on colony morphology and microscopic characters of the fungus. Hair perforation test, urease test and pigment production in corn meal agar were also

performed. Mating experiment was done on the teleomorphic identity and mating type of the isolates using the method of Padhye and Carmichael⁶. All the three strains of *T. mentagrophytes* were crossed with *Arthroderma benhamiae* RV26778(+), RV26780(-), *Arthroderma benhamiae* (African race) RV30000(+), RV30001(-), *Arthroderma vanbreuseghemii* RV27960(+), RV27961(-) and *Arthroderma simii* RV54201(-), RV25472(+) respectively. Sterilized garden soil baited with sterilized horse and guinea pig hair was used for conducting mating experiment. The plates were incubated at 26°C for six weeks away from light. Teleomorphs and mating types were identified on the basis of the production of gymnothecia. Asci and ascospores were examined microscopically.

In the present study, we isolated three strains of *T. mentagrophytes*, one each from a lion cub and two dogs. No symptomatic lesions were observed in these animals suggesting that animals may act as carriers of dermatophytes. All the animal isolates of *T. mentagrophytes* and 6/70 clinical isolates of *T. mentagrophytes* isolated in our previous study produced gymnothecia with *A. vanbreuseghemii* (-) mating type and was identified as *A. vanbreuseghemii* (+) mating type. The six clinical isolates which were recovered from severe cases of tinea capitis in

children belonged to rural Madras. These children would have contracted the disease while playing with animals. The isolation of one strain of *T. mentagrophytes* from a lion cub suggests that wild animals also may harbour pathogenic dermatophytes. Though the number of animals screened in the present study was small, findings strongly suggest that animals may act as vectors of human ringworm disease, especially in rural areas.

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Human influence on global climate

J. Srinivasan

The Intergovernmental Panel on Climate Change (IPCC) has released recently its second assessment report. This has been produced by the working group I and is called 'Climate Change 1995: The Science of Climate Change'¹. The first assessment was published in 1990 and provided an excellent overview of scientific knowledge related to climate change at that time. The most important conclusion of the second assessment is that the balance of evidence suggests a discernible human influence on global climate. This conclusion has been challenged by the fossil-fuel lobby while the environmental lobby feels that the above statement is too weak!

The major controversies in this report pertain to chapter 8 on 'Detection of Climate Change and Attribution of Causes'. In this chapter the pattern of climate change predicted by climate models (including the effect of greenhouse gases and anthropogenic sulphate aerosols) has been compared with the observed geographical, seasonal, and vertical patterns of atmospheric temperature change. There are many similarities between the patterns of observed and predicted temperature change in the northern hemisphere during the period 1963–1988 (see Figure 1). The patterns of observed and predicted temperature change are not similar in the southern hemisphere. One of the reasons for the large differences in the southern hemisphere could be the paucity of data in this region. Hence one cannot state beyond all reasonable doubt that human impact on global climate has been detected.

The most interesting aspect of the pattern analysis is the poor agreement between observations and model in the northern hemisphere if the effects of anthropogenic sulphate aerosols (from burning of fossil fuels) are excluded. Thus the most important conclusion of the second assessment of IPCC is the role of anthropogenic sulphate aerosols in cooling the atmosphere and thereby counteracting the warming by greenhouse gases. The optimists and the fos-

sil-fuel lobby may be delighted that the effect of burning fossil fuels causes both warming and cooling of the atmosphere. They may hope that these two effects may leave the earth's climate largely unchanged. This will not happen because the emission of sulphate aerosols from thermal power plants in the industrialized countries has decreased dramatically in the last decade on account of concerns related to local atmospheric pollution. If the developing countries decide to enforce their environmental pollution laws with regard to sulphate aerosols then there may be no cooling of the atmosphere. The residence time of sulphate aerosols is a few years while the residence time of greenhouse gas such as carbon dioxide can be hundreds of years. In addition, the cooling due to aerosols is confined to regions where it is emitted but that due to greenhouse gases emissions have a global impact. Hence one cannot hope for a neat cancellation of the impact of emission of greenhouse gases and aerosols by fossil fuels. Can we really trust the predictions of global warming made by the Climate Models? The answer to this question is not simple. The anthropogenic influence on global climate is small but cumulative. Hence the effect of anthropogenic emission of greenhouse gases will be seen unambiguously after hundred or two hundred years. The climate models can be tested by checking whether they reproduce the observed variation of global temperature after a volcanic eruption. In 1991, Mt Pinatubo volcanic eruption caused, for a short period, radiative changes in the atmosphere comparable (but of opposite sign) to the doubling of carbon dioxide in the atmosphere. The climate models were able to predict correctly the global cooling that occurred on account of the aerosols injected into the stratosphere by the Mt Pinatubo volcanic eruption. Hence one can have some confidence with regard to predictions made by climate models with regard to global temperature change. We cannot, however, have the same level of confidence with

regard to predictions of regional temperature or precipitation changes by climate models.

What will be the impact of global warming on account of carbon dioxide emissions by fossil fuels? The immediate impact of global warming will be a rise in sea levels. There could, however, be a large error in the prediction of sea level rise. The large error occurs because we do not know the contribution of Greenland ice sheet and Antarctic ice sheet in the sea level rise. A warmer climate will increase the melt rates at the margins in the Greenland ice sheet and cause an increase in sea level. On the other hand, a warmer climate will increase accumulation of snow (because of higher snow fall) in the Antarctic ice sheet and hence result in a fall in sea levels! A warmer ocean will, of course, cause the water in the ocean to expand but the net effect of expansion of sea water and the change in mass balance of ice sheets cannot be predicted accurately. Hence the IPCC report predicts that in the next hundred years the rise in the sea level can be anywhere between 13 cm and 94 cm. There could, however, be unpleasant surprises on account of the instability of the West Antarctic Ice Sheet (WAIS). This ice sheet rests on a bed well below sea level. The dynamics of WAIS is dominated by fast-flowing ice streams and their influence on the stability of the WAIS is still in dispute. The IPCC report concludes, however, that the likelihood of a major sea level rise by the year 2100 due to the collapse of WAIS is low.

In an eloquent article in *Nature*², Broecker has warned that unpleasant surprises may occur when the earth's atmosphere enters a phase space which it has not experienced during the last 160,000 years. Broecker has said: 'Earth's climate does not respond to forcing in a smooth and gradual manner. Rather it responds in sharp jumps which involve large scale reorganization of the Earth's system. If this reading of the natural record is correct, then we must consider the possibility that the main re-

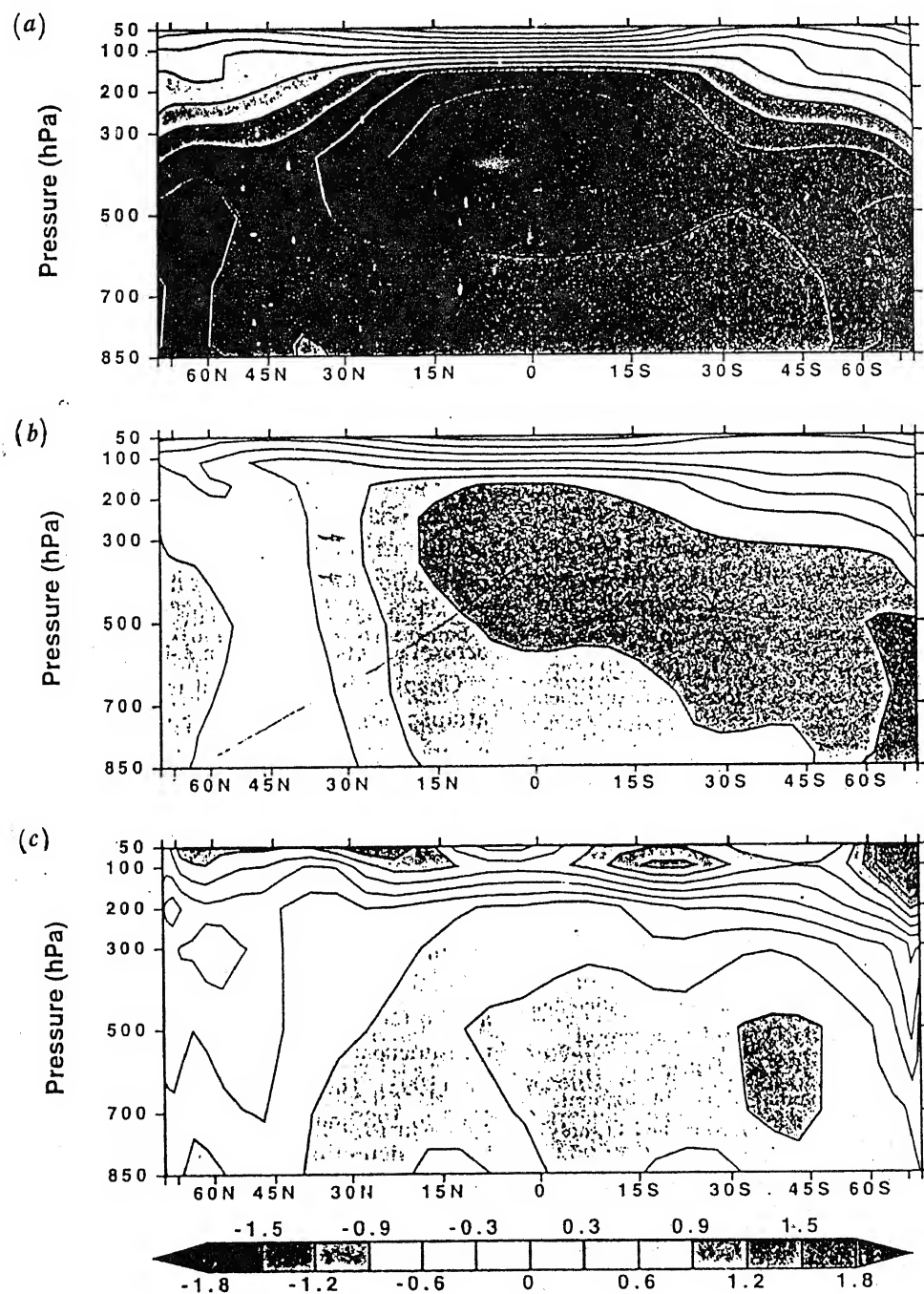


Figure 1 a–c. Modelled and observed changes in the zonal mean, annual-average temperature structure of the atmosphere. Model changes are expressed relative to a control run with pre-industrial levels of CO_2 and no anthropogenic sulphur emissions. The results obtained with present-day atmospheric concentration of CO_2 are shown in (a) and by the combined effect of present-day CO_2 levels and sulphur emissions are shown in (b), observed changes (c) are radiosonde-based temperature measurements and are expressed as total least-squares linear trends over the 25-year period from May 1963 to April 1988 (i.e. $^{\circ}\text{C}/25$ years). Figure from reference 1. For further details about the model refer to Santer, B. D. *et al.*, *Nature*, 1996, 382, 39–46.

sponse of the system to our provocation of the atmosphere will come in jumps whose timing and magnitude are unpredictable. Coping with this kind of change is clearly a far more serious matter than coping with gradual warming.' The warning with regard to unpleasant surprises should be taken seriously since the palaeoclimate record shows episodes of sudden cessation of the deep ocean circulation. There was an unpleasant surprise in 1980s when the 'Ozone hole' appeared over the Antarctic. Scientists had predicted the gradual depletion of ozone on account of the release of chloroflourocarbons (CFCs) by human beings. They were, however, unable to predict the rapid depletion of ozone that occurred in the Antarctic because the theoretical models used during that period had not incorporated the role of ice particles. The General Circulation Models (GCM) used to predict climate change are very complex and incorporate all known physical processes but one cannot rule out, for example, errors in the modelling of cloud physical processes or vegetation or uptake of carbon dioxide by the oceans.

The *Wall Street Journal* published an editorial on 12 June 1996, titled 'A Major Deception on Global Warming'. In this editorial, Fredrick Seitz (President emeritus of Rockefeller University and Chairman of the George C. Marshall Institute) has stated 'In my more than 60 years as a member of the American Scientific community, including service as president of both the National Academy of Sciences and the American Physical Society, I have never witnessed a more disturbing corruption of the peer-review process than the events that lead to this IPCC report'. He further states that nothing in the IPCC

rules permit anyone to change a scientific report after it has been accepted by the panel of scientific contributors and the full IPCC. The latter statement is not true and seems to be based on the ignorance of the rules governing IPCC reports. The first statement regarding the peer review process is unfair since the IPCC scientific report has been reviewed by more than 500 reviewers from 40 countries. As a lead author of Chapter 2 of the IPCC report 'Climate Change 1995', I was impressed by the seriousness with which every objection raised by the reviewers was discussed by the lead authors. The IPCC has made every attempt to incorporate diverse viewpoints without succumbing to the pressures exerted by various lobbies. The concern of the fossil-fuel lobby is understandable since they fear that this report may prod some countries to impose legislation that may lead to the reduction in the consumption of fossil fuels.

In view of all the uncertainty regarding the consequences of global warming, should we wait for some more time till we obtain unequivocal evidence regarding the deleterious consequences of global warming? This may mean that we may have to wait for another 50 years. The politicians and the fossil-fuel lobby will be very happy because we can postpone the unpleasant decisions to the future. The fossil-fuel lobby has been advocating this view. In the 1960s the tobacco-lobby argued that link between lung cancer and smoking was not demonstrated beyond reasonable doubt. In complex systems such as the human body or the earth it may not be possible to prove some hypothesis beyond any reasonable doubt. There could, however, be serious consequences if one

continues to smoke on the grounds that the relationship between smoking and cancer has not been proven beyond any reasonable doubt. Should we continue to burn fossil fuels because it provides us immediate benefits and not be concerned about the long-term consequences because there are uncertainties regarding the long-term effects?

The answer cannot be provided by scientists, economists, or politicians alone. In the final analysis, the answer depends upon one's attitude towards the natural world and the legacy we would like to leave for future generations. If the complex natural world is held in reverence, as most ancient people did, then one would be concerned about the irreversible changes that may be brought about by human beings. On the other hand, if one considers the natural world to be a resource to be exploited for the immediate benefit of human beings then there need be no concern for the future. In the next century, we have to strike a delicate balance between the necessity to exploit nature for the benefit of human beings and the responsibility to preserve the planet for future generations.

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Biotechnology: Its global impact and relevance to India

Harish Padh

Biotechnology industry is maturing as an economic sector in the West. Approved products in the market, over 500 therapeutic products in the pipeline and renewed public confidence make it one of the most promising areas of economic growth in the near future. With liberalized economy, India offers a huge market for these products as well as cheap manufacturing base for export. However, for a variety of reasons, India has failed in developing these technologies for commercialization and the demand at the moment is met by imports. The proper technology licensing and transfer is suggested for immediate growth in this sector. Requirement of good infrastructure including a technopark is warranted in catalysing this growth.

BIOTECHNOLOGY encompasses aspects of biology, medicine, chemistry, and engineering. It has been defined by the US Government as '... any technique that uses living organisms (or parts of organisms) to make or modify products, to improve plants or animals, or to develop microorganisms for specific uses', in other words it is Applied Biology. Although the word biotechnology is new (around 1980), the science is not. Some examples of biotechnology used in ancient times, without knowledge of the underlying microbial processes, include beer fermentation by Babylonians (6000 BC), cheese making in Iraq (6000 BC), bread making by Egyptians (4000 BC). India can also take pride in contributing to the development of ancient biotechnology in the form of septic tanks in Mohenjodaro and Harappa regions (3000 BC). Four new technologies are responsible for bringing about the recent revolution in this field: viz. (i) Genetic engineering enabling us to identify and transfer genes from one organism to another, (ii) Cell fusion technology and resultant monoclonal antibodies, (iii) Bioprocess technologies to produce large quantities of important biological drugs, and (iv) Structure-based molecular design expediting new drug development. Various combinations and derivatives of these technologies are now available. The new biotechnology is a rapidly expanding collection of tools and technologies that allow, among other things, unprecedented control over and manipulation of the genetic material of organisms. In a short time, its impact is already visible, while its long-term potential is still unrealized at the moment. The book *Jurassic Park* by Michael Crichton and the movie by Steven Spielberg helped educate the general populace about the potential of genetic engineering.

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What can be done with these technologies?

Human body is a chemical factory running thousands of chemical reactions at a time. These reactions are catalysed by proteins which are made from respective genes. Therefore, a state of our body, healthy or otherwise, is determined by coordinated proper functioning of these proteins and genes. Recent advances have made it possible to isolate these genes and transfer them to another individual. In addition, biotechnology provides us ability to make rare but important proteins in abundant quantity. These advances have made it possible to use these genes and their protein products for therapeutic and diagnostic purposes. Unlike chemicals, these biologicals can be used for more effective treatment of various ailments, specially those for which there are no cures available at the moment. These possibilities also offer tremendous opportunities to improve agriculture products, animal stock, and to correct genetic disorders.

New biotechnology and its impact

The potential of biotechnology is responsible for bringing about the current revolution in biology. We now have in our grasp the means to produce large quantities of rare, medically valuable molecules, to modify plants and animals to carry specific hereditary traits, and novel means to detect disease, produce useful chemicals, and clean up and restore the environment. Thus, biotechnology has profound impact in the fields of health, food/agriculture and environmental protection. It has the potential to provide a wide array of benefits to humanity, including treatment for previously incurable diseases, healthier dairy and agricultural products, more resilient and productive crop plants and increased production of renewable sources of energy.

The US industrial sector now includes more than 1200 biotechnology start-up firms. More than 200 established companies, including many major chemical and pharmaceutical firms, have diversified into biotechnology. The distinction between biotechnological and pharmaceutical sector is fading because of the heavy involvement of pharmaceutical firms in biotechnology business. In little over a decade, this nascent industry has grown to be a multibillion dollar sector. By the end of the century this sector will match or surpass the computer industry in size, importance and growth.

Health care

Health care to-date has utilized biotechnology the most. The primary reasons are lack of good alternatives for treating the sick, dramatic results, big profits and good publicity. We still have many ailments for which we have no treatment or cure, for example, numerous genetic diseases, Alzheimer's disease, many types of cancer, hypertension, and AIDS. Biotechnology has allowed use of previously unavailable biologicals as medicines and made it possible to produce therapeutic biologicals in large quantities. Biologicals are our own molecules and are unlike chemical drugs. Biotechnology-derived proteins and polypeptides form the new class of potential drugs. For example insulin, used in the treatment of diabetes, was once primarily extracted from slaughtered animals. Since 1982, human insulin (Humulin®) has been produced by microorganisms in huge fermentation tanks. The global sales of Humulin® and other biotechnology drugs like Epogen® (Epoetin alfa from Amgen) and Recombivax HB® (Hepatitis B vaccine from Merck) have reached around US\$ 1 billion each. These products are convenient to make and more compatible with biological systems. In the recent past, the discovery of conventional drugs (heterocyclic chemical drugs including antibiotics) has slowed down. The number of biotechnology-derived new protein drug candidates has surpassed new chemical drugs since 1987 (see Figure 1).

At present there are about 35 biotechnology-derived

therapeutics and vaccines approved by the US FDA for medical use and over 500 additional drugs and vaccines by 150 companies are in various phases of clinical trials. Biotechnology has also spurred growth in diagnostics and over 600 biotechnology-based diagnostics are now available in clinical practice.

Gene therapy offers the potential to correct diseases at the genetic level. After creating scores of genetically modified plants and animals, human gene therapy looms on the horizon. The year 1995 marked significant developments in gene therapy. In USA there are about 130 approved gene therapy protocols. Half of these protocols are active with a total of about 600 patients under trials.

Agriculture

Although this area has benefited most from the ancient biotechnology, food/agriculture has been slow to utilize the modern advances in biotechnology. The reasons for this are numerous: a plentiful supply of food in the Western countries, the availability of other alternatives, apprehension over new technology, the complexity of plant and animal genomes, etc. The potential applications of biotechnology in foods and agriculture include better management of agro-ecosystems through decreased use of chemicals, increased use of pest and herbicide-resistant plants, new methods of maintaining soil productivity, better water management and new healthier foods with increased societal value. Functional foods (neutraceuticals) represent an active area of research to increase the nutritional value of foods, prevent diseases, and reduce healthcare costs. At present there are 14 or so sources of phytochemicals known to fight or retard malignancies. Biotechnology in the food/agriculture area is breaking new ground. Current public debate about BST (bovine somatotropin, a hormone administered to cows to increase milk production) typifies an example of a biotechnology product testing public acceptance. Similarly, the FlavrSavr™ tomato (produced by transgenic plants engineered by antisense technology to preserve flavour, texture and quality) is an example of a new breed of value-added foods. Food biotechnology offers valuable and perhaps the only viable alternative to food problems of developing countries. Food biotechnology also offers workable solutions to nutritionally influenced diseases like heart diseases, hypertension, arthritis, cancer, and diabetes.

Environment

Biotechnology products, like other 'high-tech' products, are inherently resource conserving, especially in comparison with older industrial methods of production.

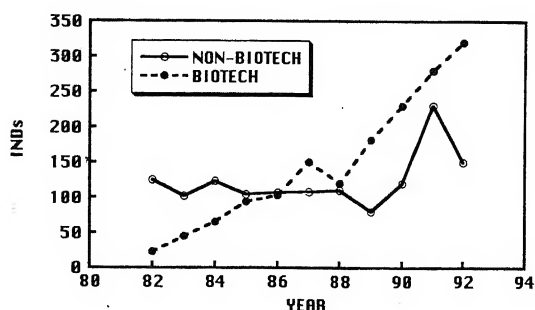


Figure 1. Investigational new drugs: Biotechnology vs non-biotechnology.

GENERAL ARTICLES

Biotechnology offers additional environmental advantages when applied to current and emerging manufacturing processes. When applied to environmental issues, biotechnology is enhancing the pre-existing ability of nature to degrade compounds – disposing of wastes in a very *natural* way.

In March 1989, the oil supertanker Exxon Valdez lost 33 thousand tons of oil off the coast of Alaska at Prince Williams' Sound. For the first time, bioremediation technologies were employed in an actual oil spill to clean up crude oil contamination at the site of spillage. Several of the field tests were highly successful, with the added advantage that subsurface contamination (contamination below the surface of the soil or beach) was also remediated up to a depth of several feet.

Economic impact of biotechnology

Biotechnology companies develop technologies and products that expand the boundaries of medicine, agriculture, industrial processes and environmental science. They also build sustainable companies that bring new ways of competing and succeeding into traditional industries. The following are the highlights of biotechnology business in the USA. The European market is roughly of the same size as that of USA and no information is available for the market size in other continents.

General business highlights

- In Europe and USA, the sale for biotechnology-derived therapeutics is estimated to be 15 billion US dollars (Rs 52,500 crore). The sale of diagnostics is estimated to be another 15 billion US dollars. It is a multibillion dollar sector. Market size in the developing countries is not known. In USA, biotechnology has captured 10–15% of the total healthcare market.
- The sales, revenues, and equity in this sector are increasing with an annual rate of about 10–20% (Table 1). With many products in the pipeline and increasing public education and acceptance of biotechnology, the momentum will continue.
- Table 2 shows ten years history of biotechnology.
- It is a R&D-intensive sector (Table 1). At the moment, much of the revenues are poured back into R&D, compared to about 6% invested in R&D by US pharmaceutical sector and about 1% of sales invested in R&D by the Indian pharma sector.
- Total public equity raised is about \$1 billion a year (Figure 2). Some decline since 1992 has been reversed since 1995.
- Financing (raising capital) varies from year to year averaging about \$2 billion per year contributed almost equally by public offering, venture capital and private

Table 1. Highlights of biotechnology industries in USA (billion US\$)

	1995	1994	Per cent change
Sales	\$9.3	\$7.9	18%
Revenues	\$12.7	\$11.3	12%
R&D expenditure	\$7.7	\$7.1	8%
Market capitalization	\$52.0	\$41.0	27%
Number of companies	1,308	1,311	0%
Employees	108,000	103,000	5%

Table 2. Ten-year history of the biotechnology therapeutics in USA

	1985	1990	1995
Sales (\$ in billion)	\$1.1	\$2.9	\$9.3
Revenues (\$ in billion)	\$2.2	\$4.7	\$12.7
R&D expenditure (\$ in billion)	\$1.7	\$2.6	\$7.7
Number of companies	850	1,107	1,308
Employees	40,000	66,000	108,000

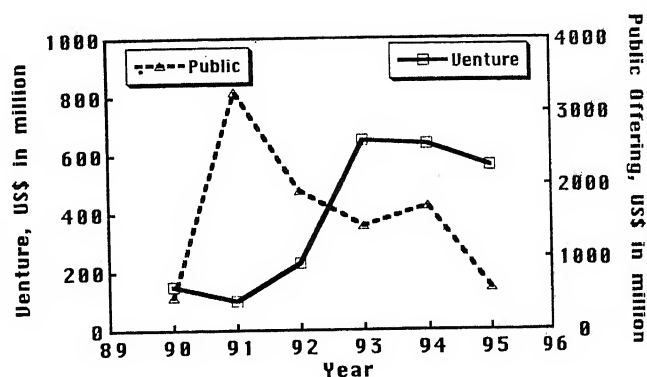


Figure 2. Biotechnology financing.

placement. Total money raised in 1995 through venture, private placements, initial public offerings and followups and strategic alliance was about 3.5 billion US Dollars.

- Agriculture biotechnology is expanding with a total market value of \$600 million with Calgene, which introduced FlavServ tomato, leading the market with \$200 million.
- Many genetically modified plants and plant products have obtained 'deregulated' status in USA and Europe, meaning no special approval necessary from FDA or USDA.
- The Biotech Equity Index (by Coopers and Lybrand, CLBI) has done better or as good as Standard & Poor 500 (broad-based equity index) over the period of time.
- Figure 1 shows that most of the new drugs coming to the market are biologicals manufactured by biotechnology.
- Biotechnology is entrepreneurial in nature. Over 90% of the biotech companies are small or medium size.

The larger companies are now entering the area by merger and acquisition.

- In later half of 1995, the stock market has come around for this sector and biotechnology is viewed as a promising area.
- Recently, European countries in general and Germany in particular have become receptive to biotechnology.
- Global market for industrial enzymes was about 1825 million US \$ in 1995 (Table 3) and increases about 20% a year.
- Top ten biotechnology companies are: Amgen, Genentech, Chiron, ALZA, Biogen, Genzyme, Genetics Institute, Centocor, IDEXX, Immunex.

Healthcare sector – Business highlights

- The Healthcare sector has so far benefited the most out of biotechnology. The Agriculture and Environment sectors are picking up. Among healthcare companies, therapeutic is followed by diagnostic.
- There are about 35 biotechnologically-derived therapeutics in the market in USA and Europe. About 500 more products by 158 companies are in human clinical trials. Many of these products will be in the market in coming years (Table 4).
- Human Genome Project, a mammoth undertaking to get a complete blueprint of human genetic material,

Table 3. Global market for industrial enzymes

User industry	1995, US\$ in millions
Detergent	710
Starch	220
Textile	245
Others	650
Total	1825

Table 4. Biotechnology pipeline

Status in clinical trial	Phase I	Phase II	Phase III	Approved
No. of therapeutic products	144	205	127	33

is viewed with tremendous potential for future products. Many established pharma companies paid handsome price for biotech firms with expertise, technologies or products from this project.

- There are over 600 new biotechnology-based diagnostics approved for use. Most of them are based on monoclonal antibodies (Table 5).
- Table 6 lists major biological therapeutics in use. Their sales are multibillion dollars each.
- Table 7 lists some of the recently approved biological therapeutics.

Agriculture and environment sectors – Business highlights

- Some of the applications of biotechnology in agriculture are in biopesticides, animal growth hormones, genetically modified plants and animals, animal and food diagnostics, animal vaccines, etc.
- *Pharming* is a new concept where therapeutic drugs are produced in the farm animals. For example, therapeutic proteins secreted in goat milk. There are about half a dozen companies specializing in this technology to make products like lactoferrin, tPA, hemoglobin, melanin and interleukines in cows, goats and pigs.
- Biopesticides are coming to the market and their sales are increasing.
- Bioremediation, which is environmentally friendly approach to clean the environment, is projected to reach global sales worth of 1 billion US \$ by the year 2000.

Future prospects for biotechnology industry

The biotech industry is just coming out of its infancy. Its potential is being tested, realized and used. The public awareness and acceptance will accelerate the process. This sector is expected to expand at least 3-fold by the end of the century and will match or surpass the computer industry in size, importance and growth (Table 8). It holds a good promise in a number of areas, specially those for which presently we have no treatment. There is tremendous potential for developing countries like India to apply biotechnology for agriculture and environmental resources.

Table 5. Approved biotech diagnostics

Type	Infectious disease	Tumour marker	Analyte and drug	Blood screening	Total
Monoclonal antibody	127	2	433	9	571
DNA probe	42	0	11	0	55
Recombinant DNA	11	1	1	0	13
Total	180	3	445	9	637

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Table 6. Biotechnology-derived drug products

Products	1996 sales (billion US\$)	Selected manufacturers
Human insulin	2.3	Eli Lilly, Novo Nordisk, Hoechst, Yamanouchi
Growth factors	2.0	Eli Lilly, Novo Nordisk, Genentech, Pharmacia
Blood factors	5.0	Amgen, J&J, Sankyo, Chugai, Sandoz, Immunex
Interferons/interleukines	3.0	Schering-plough, Roche, Sumitomo, Wellcome, Daiichi
Monoclonal antibodies	0.4	J&J, Cytogen
Vaccines	1.0	SmithKline, Merck, Shionogi
Others	1.0	Genentech, Baxter, Bayer, Genzyme

Table 7. Examples of new biotechnology derived drugs recently approved

Company	Drug	Indication
ALZA	DynaCirc CR	Antihypertensive drug
DNX	Biodigm	LDL reduction
Quadra Logic	Photofrin	Photodynamic therapy
Amgen	Neupogen	Severe neutropenia (new indication)
Centocor	ReoPro	Anti-platelet for angioplasty
Immunex	Thioplex	Cancer
NeXstar	DaunoXome	AIDS-related Kaposi's sarcoma
U.S. Bioscience	Ethylol	Prevent kidney damage by cisplatin

Table 8. Comparison of biotech and computer industries

US biotechnology industry	US computer industry
Young	Maturing
Technology intense, R&D critical	Technology intense, R&D critical
Heavily regulated	Unregulated
Capital intensive	Moderate capital requirements
Global market	Global market
Long product development timeline	Short product development timeline
Annual sales of \$9.3 billion	Annual sales of \$80 billion
Number of companies 1,308	Number of companies 2,134
Number of employees 108,000	Number of employees 350,000

Biotechnology in India

- India has a number of good academic institutions with expertise in basic sciences relevant to biotech-

nology. India also has a fair number of suppliers and stockists who market required reagents and supplies. Assuming that the academic-industry interaction will improve, India is poised to explore biotechnology for business.

- Their is a surge in testing facilities, technology transfer, R&D institutions, small equipment fabricators, repackers and sellers of imported materials, etc.
- Proliferating private medical care and its importance in creating demand for biotech products.
- Catching up with the Western way of healthcare.
- India has rapidly swelling population of upper and middle class which has created unmet demand for newer diagnostics and therapeutics.
- Proliferation of diagnostics – mostly imported.
- General failure of diagnostics (imported as well as locally developed).
- Pharmaceutical industry in India is very strong and vibrant with expertise for chemical drugs. It has little experience in biotech diagnostics and no experience in biotech therapeutics.
- India is competing well in bulk drug production market. This is largely due to the more efficient nature of the processes and manufacturing costs. The same can hold true for the biological drugs.
- India's economic liberalization and signing of GATT and DUNKEL Draft clears the way and need for significant R&D activities by pharmaceutical industry.
- Impending patent changes will make it necessary to either develop our own technology or obtain proper license from others.
- Globalization of economy and liberalization of our economy make this an appropriate time to seek outside licensing and technology transfer.
- R&D activity in pharmaceutical industry will surge but with no significant experience in biotech, the pharmaceutical industry will need help.
- Local problems (malaria, tuberculosis, etc.) have remained untouched by biotechnology either in India or overseas. These problems will need innovative approaches.
- Agriculture, seed technology and environment could benefit tremendously by use of biotechnology.
- Table 9 indicates various factors influencing growth in a particular segment of economy. For example, in USA the growth is driven by innovation while in Germany the growth is driven by capital investment and proper technology management. In places like Thailand (and India?) the factors like cheap labour or availability of raw materials, etc. can spur growth. In a place like India and China, sudden growth is evidenced simply because of the rapid opening of huge consumer market. These and other factors lead us to believe that India is poised to enter biotechnology market.

Table 9. Driving forces influencing industrial growth

Driving force	Precondition	Strategy	Example
Factor	Factor advantage	Low tech sectors	Thailand, India???
Investment	Mature user industry	Investment in getting foreign technology, joint government and private ventures	Germany
Innovation	Developed science base, mature industry base, venture capital	New companies led development	USA
Market base	Sudden opening of large consumer market	Careful import and promotion of proven technologies	India, China, Eastern Europe? Former Soviet countries

- Infusion of foreign technologies and collaboration has already taken place in other sectors of economy. It is now time for Biotech/Pharma sector to seek proven technologies from outside.
- Import of technology will be a necessity. Government should provide proper channel and infrastructure to the pharma industry. This will lead to value added better stable, tested and validated products in the market.
- Other governments, including Germany has government-supported agencies for technology identification and import.
- Pharma industry is located between Mumbai and Ahmedabad (90% of the drug production in India is in Gujarat and Maharashtra). There is no government institution or university in this region with expertise in this area to help pharma industry.
- State industrial development corporations (SIDCs) should get serious about this sector and promote this sector by providing infrastructure and expertise.

Indian pharma industry

Indian pharmaceutical industry, with an expertise in chemical drugs, has done excellent job in the recent past. Total pharmaceutical industry sales is estimated to be Rs 50 billion. India is competing well in bulk drug as well as formulation markets. This is largely due to the more efficient nature of the processes and reduced manufacturing costs. The same can hold true for the biological drugs once the industry enters into manufacturing of biologicals. Moreover, India's economic liberalization and signing of GATT and DUNKEL Draft clears the way and need for significant R&D activities by pharmaceutical industry. Therefore, for a variety of reasons, the Indian pharmaceutical industry will sooner or later enter in manufacturing of biotechnology-based diagnostics and therapeutics.

Western India (Gujarat, Maharashtra and Rajasthan)

with more than 50% of the registered pharmaceutical units accounts for 90% of pharmaceutical production. The region also accounts for more than 70% of import and export of the pharmaceuticals.

Diagnostics

Worldwide there are about 600 new biotechnology-based diagnostics in the market with a value of about 20 billion US\$. Many more are about to enter the market, the most prominent among these will be PCR-based diagnostics. In India the diagnostics sales are expected to be between Rs 1 billion and Rs 2 billion. India relies on imports for many of the immunodiagnostics kits. Many of the locally developed diagnostics have failed, while the imported diagnostics are either unsuitable or expensive.

Therapeutics

Expression of foreign genes in convenient prokaryotic cells and the large-scale production of gene products is now routine. These protein products could have applications as therapeutics, diagnostics, restriction enzymes or industrial enzymes. At present, there are about 35 biotechnology-derived therapeutics approved for human use in USA. The total market value of these products is about 50 billion US\$. About 150 companies have 490 more products in various stages of clinical trials and development. With increasing acceptability of biotech products, there will be about 200 biotechnology-derived therapeutics available in the market by the turn of the century. In 1987, the number of new drugs produced by biotechnology has overtaken investigational new drug (IND) produced by conventional means (chemical and antibiotic drugs). This is an indication of the trend that in future new therapeutics will be made by cellular factories through recombinant technologies.

In India, there is no locally manufactured recombinant

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therapeutic product available in the market. Few imported biological therapeutic products are marketed in India, e.g. human insulin and streptokinase.

Need for licensing and transfer of technologies

We have witnessed that indigenously developed diagnostics have shown less than satisfactory performance in the market. The imported diagnostics are not suitable because they are expensive, not directed against local pathogenic strains, and with little or no quality and stability controls. In addition, the Indian pharmaceutical industry has little or no experience in modern diagnostics or biological therapeutics. Their approach of process improvisation that worked well for chemical drugs will not work in biotechnology-based diagnostics or therapeutics. In addition, the number of newer diagnostics in the international market every year is burgeoning. Therefore, the national interest is better served by a systematic approach in identifying and transferring and licensing of appropriate technologies.

In the international market, biotechnology drugs are very expensive. For example, Genentech's tPA is priced at about \$2,000 per injection and streptokinase is marketed at about \$200 per injection. A genetically engineered Factor VIII used in the treatment of hemophiliacs cost \$25,000 a year. The next generation of these products will have to be less expensive and more effective. India can provide inexpensive manufacturing base for Indian as well as export market. Imported therapeutics traded in India are exorbitantly priced (Rs 300 per dose of human insulin compared to Rs 65 per dose of traditional insulin) and about Rs 4000 per dose of streptokinase. What will be our strategy when in five years there will be over 200 therapeutics and vaccines available? Shall we still rely on imports?

Need for technology development

There are a few diagnostics developed in the country but overall performance has been dismal. There seems to be a gap of culture, communication or something between the academic and corporate worlds as a result of which the indigenous technologies have not been

developed. The products do not reach the market or fail in the market. For a long-term interest of the nation there is a need for local development of technologies, especially against tuberculosis and malaria.

Agriculture

India which can use biotechnology for agriculture and environment cleaning has not really begun to exploit biotechnology in these areas. We have not produced genetically altered plants or engineered microorganism for bioremediation. The only activity India has developed is in plant tissue culture and plant micropropagation. In this sector, entrepreneurs have by and large imported technologies from abroad (mostly northern European countries) with buy-back arrangement for exporting the plant products. Locally developed technologies have found very limited use in the market. Local demand for these products is also highly underdeveloped.

Summary

We have to stop relying on imports and think about local manufacturing of the products mentioned above. This can be best achieved by proper technology licensing and transfer. We should also focus on technology development for the diseases of the developing countries.

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The non-organic theory of the genesis of petroleum

Samar Abbas

Recent advances in interdisciplinary fields as diverse as astrophysics, cosmogeophysics, nuclear geology, etc. have led to interesting developments in the non-organic theory of the genesis of petroleum. This theory, which holds that petroleum is of an abiogenic primordial origin, provides an explanation for certain features of petroleum geology that are hard to explain within the standard organic framework. If the non-organic theory is correct, then hydrocarbon reserves would be enormous and almost inexhaustible.

PETROLEUM is the foundation of the industrial civilization. It is from petroleum that the world obtains its chemicals, fuel for automobiles, engines, airplanes, etc. and its energy supply for its power stations. Empires have risen and fallen due to the annexation or loss of oil fields. Hence, the origin of petroleum and the assurance of future energy supplies is of the utmost importance if this world is to continue as it is. It is generally believed that currently recoverable crude oil reserves will be nearing exhaustion within a few decades. This estimate is based on the conventionally accepted (organic) theory of the origin of petroleum.

However it does not necessarily follow that this civilization will fall into darkness. The origin of petroleum still, despite the immense amount of research devoted to it, has more uncertainties concerning it than any other common natural substance¹.

There are two basic frameworks: the standard organic theory, and the non-organic theory. The former holds that petroleum is of an organic origin and is the currently favoured proposal. It predicts limited reserves worldwide; moreover Indian reserves are predicted as minimal. The latter maintains that it is of non-organic genesis, supposedly of primordial origin. On the basis of this theory, oil resources would be much larger than those predicted by the biogenic theory. India, oil-poor in the biogenic framework, is predicted to be oil-rich in the non-organic one.

Unfortunately the abiogenic theory and its implications are not well known. Moreover, both opposing sides have taken uncompromising, even fundamentalist views on occasions. There is hence a crucial need, especially for nations such as India, to objectively assess the situation and investigate the latter possibility more carefully; especially since, as we shall discuss below, the evidence

in favour of either candidate is inconclusive and the question still remains an open one.

The organic theory

Outline

The organic theory holds that the first stage of the genesis of petroleum involves plankton (single-celled organisms that float on the oceans). These die and gradually accumulate on the ocean floor. Other sediments start accumulating too, and after a few million years the plankton are buried under several km of sediment. The plankton, which have remained unoxidized, under the increased values of pressure and temperature, are now transformed into kerogen. Under favourable conditions of time and temperature this kerogen, after further burial and heating, is transformed, via cracking, into petroleum and natural gas. These then migrate towards the surface and end up either reaching it (and drying up to yield bitumen or tar) or being arrested on the way in traps (where, millions of years later, drillers of the present industrial age make their big strikes)².

Advantages

Traditionally, the following points have been considered as supporting the biological theory:

- (i) Since it is known that hydrocarbons can be produced by photosynthesis, it is natural to expect petroleum to be of an organic origin.
- (ii) Molecules thought to be of biological origin, e.g. porphyrins, isoprenoids, hopanoids, etc. were found in petroleum, thereby providing support for the organic theory.
- (iii) The organic carbon in plants is depleted in carbon-13 due to the process of photosynthesis. In dead organic

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material the C-13 is further depleted due to radioactive decay. Since it was found that most petroleum and natural gas showed the same depletion, it was viewed as a strong proof in favour of an organic origin.

(iv) Sediments are the most important host rocks yielding petroleum, i.e. the oil produced from oil wells is generally obtained from a porous sandstone deep below. Often sediments are associated with biological material that could have acted as a source of the petroleum.

(v) The existence of large quantities of oil shale from which a hydrocarbon mix similar to petroleum could be distilled was seen as a support in favour of an organic origin. This followed easily, since the oil shale was taken to be the kerogen source rock which, on sufficient burial, purportedly yielded petroleum.

Disadvantages

However the following observations go against the organic theory:

(i) The discovery that meteorites contain hydrocarbons came as a great blow to advantages no. (i) and (ii) of the organic theory. Porphyrins and isoprenoids have been found on meteorites³. In addition, the outer planets contain large amounts of hydrocarbon.

(ii) The concentration of oil in the Middle East implies that that region must have been exceptionally prolific in plant and animal life over long periods of the Earth's history. This is unlikely, since life tends to be more dispersed, even today.

(iii) The biological supports of optical activity and an odd-even effect disappear at low levels. There is a sharp cutoff to the effect of optical activity: petroleum in Philippi's study⁴ was found to be optically active if derived from a reservoir with a temperature below 66°C, but surprisingly petroleum from deeper levels of the same field did not exhibit the phenomenon of optical activity. Gold⁵ proposed that a certain bacteria ceases action above 66°C, but he unfortunately did not suggest any candidates.

(iv) Methane occurs in giant ocean rifts, in continental rifts and the lakes that occur nearby, e.g. dissolved in the waters of the East African Lake Kivu⁶, as methane hydrates in permafrost, in active volcanic and mud volcanic regions, as well as at great depths of more than 10 km as geopressed gas etc. A biological origin for this methane can be virtually ruled out.

In the light of these difficulties one should consider the other rival non-organic theories as serious possibilities. They forecast much larger oil reserves than previously imagined and that too in regions which, according to the organic theory should be devoid of all petroleum.

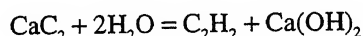
The non-organic theory

Historical development

The non-organic theory of the genesis of petroleum has a long history, dating back to the early days of the oil industry. Its development has led to the birth of a number of variants, the most important of which are outlined below:

Metal carbide theory. The founder of the non-organic theory was Mendeleev, the Russian chemist who proposed the modern version of the periodic table. In 1877, he wrote that the petroleum deposits of the world seem to be controlled more by large scale tectonic features than by the ages of sedimentary rocks⁷. To explain these observations he put forth the metal carbide theory. Many contemporary investigators, mostly Russian, supported Mendeleev's view.

In this model metal carbides deep within the earth reacted with water at high temperatures to form acetylene which subsequently condensed to form heavier hydrocarbons (as is readily formed in the lab). The following reaction



is still popular amongst some astronomers and certain Russian geologists as a major petroleum-forming possibility².

Nebular condensation theory. In 1980 Sokoloff proposed that 'bitumina', at that time meaning the whole range of hydrocarbons from petroleum to tar, precipitated as rain onto the newly forming earth from the original nebular matter from which the Solar System was formed. In modern terminology he simply suggested that petroleum originated from meteorites. Later, he claimed, this petroleum was ejected from the earth's interior into the surface sediments⁸. Recently this idea has been supported by Hoyle, who proposes that not only oil, but life itself has extra-terrestrial origins⁹.

Volcanic origin theory. This postulate involves outgassing of the mantle via volcanic activity¹⁰.

Earthquake outgassing. This theory proposes that outgassing occurs via deep faults, and that this is still occurring today. The detailed mechanism has a long history. Vernadsky propounded the notion that hydrocarbon compounds would be stable against dissociation and oxidation at great depths and would replace carbon dioxide as the chief carbon-bearing fluid. Kudryavtsev set forth the observations supporting what was later to be known as Kudryavtsev's rule (to be discussed shortly).

Gold^{5,11} has become the main proponent of the idea of a non-organic origin in the West. Due to his initiative, a hole was drilled into crystalline basement rock which Gold predicted should yield petroleum. Only noncommercial quantities of petroleum, if any, were found. Moreover most of Gold's colleagues were not convinced. However, recently a nearby hole did strike oil (see below). This is now the most commonly known variant.

However, since Western nations either possess or control much of the world's petroleum reserves, there is no incentive to innovate. Since the nonorganic theory predicts petroleum in much larger quantities and in areas hitherto considered unfavourable, it is petroleum-poor countries like India who stand most to gain. Hence, it is they who should take the risks of exploring the non-organic theory, both theoretically and experimentally.

These are the prime variants of the theory. From now on, the word 'non-organic theory' shall be taken to propound merely a primordial (i.e. dating from the birth of the Earth) origin of petroleum which has been migrating outwards from great depths of the Earth to form all hydrocarbon deposits from tar and tar sands to oil shale. The detailed mechanisms mentioned above shall not complicate the issue during the course of the following discussion.

Outline of the theory

The theory suggests that most of the hydrocarbons on earth are in fact primordial. Carbonaceous chondrites appear to have been the most abundant source rock during the formation of the earth. This type of meteorite contains a significant amount of hydrocarbons. As the earth formed, it would have acquired these hydrocarbons via accretion (bodies of roughly equal size clumping together through collisions), and later through meteorite impacts (including hydrocarbons formed by the reaction of meteoritic carbon with H_2 at high pressures and temperatures on impact). Then as the earth gradually cooled, a solid crust developed, while the interior remained liquid or semisolid. The volatile substances would be expelled from the interior. It is such gases that yielded, after biological modification, the present atmosphere. That hydrocarbons are being evolved from the inner parts of the earth is evident from the presence of mud volcanoes, flames seen during earthquakes, etc. On the way up, it is supposed, the oil (dissolved in methane) would be trapped in suitable formations creating the world's oil and gas, tar sands, oil shales, bitumen, mud volcanoes, etc.¹¹ Kropotkin and Valyaev¹² pointed out that the hydrocarbons, carried upwards by streams of compressed gases, would have two possible destinies: (i) In volcanic regions, they would be oxidised to carbon dioxide and water, and (ii) In 'cool' regions the hydrocarbons would form oil and gas reservoirs after con-

densation from the rising stream at levels possessing the requisite values of temperature and pressure.

Evidence in favour of the non-organic theory

Geographical location. The major oil fields of the world are concentrated on or near belts of major tectonic activity or in fact along fault zones. Some of the phenomenal Arabian fields, the world's largest petroleum province, lie along the Persian Zagros Mt. belt. The large North Sea reserves that have made much of Northern Europe self-sufficient in oil production lie along the North Sea trench. The oil fields of Indonesia and Burma closely follow the seismic belt running from New Guinea to Burma, while the oil fields of Gujarat appear to be associated with the Cambay fault. Hydrocarbons are found in the Red Sea rift Valley, the East African rift and the eastern branch of the Pacific rift. These and many other examples that exist should illustrate the association of hydrocarbons with large deep-seated cracks in the Earth's crust rather than any local sediments. However, note the idea of deep-seated cracks may also be required to explain the migration of petroleum within the organic theory.

According to the non-organic theory, petroleum should occur universally in areas of tectonic activity. This does not appear to hold true, and this seems to be a problem for the non-organic theory.

Multilevel fields. It is observed that petroleum, in at least small quantities, is often present in horizons below many accumulations, largely independent of the composition and mode of formation of the horizon. This is known as Kudryavtsev's rule, and several examples of it have been noted¹³. The suggestion that the petroleum seeps from underneath is supported by the evidence of fractionation, although this can also be explained by migration from deep source rocks within the organic framework as well.

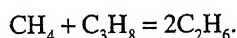
Methane-bearing strata in the same column show a progressive depletion in the isotope carbon-13 as one rises from lower levels to higher levels¹⁴. The organic theory holds that petroleum originating from source rocks buried several km within the earth explains these properties. The oil and gas formed would migrate upwards, thereby explaining both fractionation and Kudryavtsev's rule. However, this effect would be a natural consequence of the upward migration of primordial gases, with the heavier isotopes of carbon rising more slowly than the lighter one.

Stability with depth. It was once thought that petroleum and natural gas would not be able to survive at depths greater than a few tens of kilometres below the surface as the temperatures occurring there exceeded those

observed to destroy petroleum and natural gas in labs. Hence, it was reasoned, it was pointless to look for either the fuel or its origin in the depths of the Earth. However that picture has changed radically. Huge quantities of gas have been discovered at great depths, e.g. in the Anadarko basin in Oklahoma. Reservoirs of 'geopressed gas' have been found to underlie all major oil-bearing regions. These are sandstones and shales containing enormous amounts of gas dissolved in salt brines. Reserves of such gas are estimated at 60000 TCF (trillion cubic feet) in the US alone¹⁵, exceeding by several factors the total conventional gas reserves of the world.

In addition, vast domains of gas exist in open fractures of non-sedimentary basement rock. A deep hole currently being drilled in Germany has found these at depths of up to 4 km¹⁶. Theory had to be revised, and Chekaliuk¹⁷ showed that not only could natural gas exist at extreme depths but petroleum could, too. The earlier experiments had simply not been done at the correct pressures. In fact, the pressures encountered stabilize oil and natural gas against dissociation despite extreme temperatures, so that methane could exist up to 30 km with only 5% dissociated. Further, thermodynamic calculations show that petroleum itself is mostly stable between 30 and 300 km. Although this is heartening from the perspective of the non-organic theory, this does not necessarily go against the organic theory, as we discussed earlier.

Sugisaki and Nagamine¹⁸ have recently investigated the thermodynamic equilibrium of light hydrocarbon gases. Thermodynamic equilibrium of a gas is revealed by the concentrations of its constituents. They studied the reaction



At equilibrium the graph of $[\text{CH}_4] \times [\text{C}_3\text{H}_8]$ vs $[\text{C}_2\text{H}_6]$ is a graph of constant slope. Moreover, the temperature of equilibrium can be calculated from this graph. It was found that hydrocarbon gases released by crushing plutonic rock and natural gas from deep wells displayed these features, indicating chemical equilibrium. On the other hand, gases issuing from a peat bog, shallow gas wells, and, significantly, pyrolysis products from kerogen, coal and other organic substances did not, although the temperature was 350°C (thus exceeding the equilibrium temperature of 180°C for the plutonic gases) and the longest experimental period was 555 days. Quite naturally this is a puzzle, since if kerogen were the source of petroleum, then the hydrocarbons released through pyrolysis of kerogen should display the chemical equilibrium shown by the deep-level hydrocarbons. It should be noted that this work¹⁸ pertains to thermodynamic equilibrium of light hydrocarbon gases and not to the stability of the same.

The team explained these observations in terms of the organic theory as follows: After the decomposition of petroleum, kerogen and other heavy hydrocarbons, the gases attain chemical equilibrium at the high temperatures existing at great depths. As the gas cools, either by upward migration or by cooling of the volcanic rock (in the case of the plutons), the gas composition is frozen in once the temperature becomes so low that the gas composition effectively freezes in. To explain the negative result, i.e. the lack of equilibrium of the pyrolysis products, they are forced to make the rather unilluminating assumption that the rate of reaction was so slow that equilibrium could not be attained even after $1\frac{1}{2}$ years.

A far more natural explanation, which the authors cited above chose not to investigate, is using the non-organic theory. If it is assumed that the hydrocarbons are primordial and originate from the depths of the earth, then they would naturally display the signatures of chemical equilibrium as it would have been subject to high temperatures for a much longer time. As the hydrocarbons migrate upwards, they would, at shallow levels, be invaded by bacteria, thereby losing the signatures of equilibrium.

Critics suggest that oxidation would destroy any petroleum anyway turning the hydrocarbons into CO_2 , H_2O and coke, the constituents of volcanic gases. Since these come from deep inside the Earth, the primordial hydrocarbons, even if they existed, would have been destroyed. However that is not the full story. Substantial evidence indicates that unoxidised carbon exists at great depths. Unoxidised carbon can also exist at great depths within the organic framework, coke being produced by the decomposition of hydrocarbons.

Existence of unoxidised carbon at great depths

The following evidence suggests that large masses of unoxidised carbon exist at great depths:

Diamonds. These provide evidence of the existence of carbon at great depths in unoxidised form, i.e. other than CO_2 , since diamond is pure carbon.

The diamonds might have been associated with extremely violent explosions, since if the diamonds had risen slowly along with magma of the type spewed out by conventional volcanoes (e.g. Hawaii), they would have been transformed into graphite. As thermodynamical calculations and experience in the production of artificial diamonds show, diamonds must be cooled quickly to exist. Moreover the associated host rock contains other high pressure minerals like peridotite (believed to be one of the principal constituents of the mantle), etc. The primary deposits of diamonds are the rare kimberlite pipes, named after the now legendary South African

town of Kimberley which was once the chief source of these stones. These are deep vertical shafts, funnel-like near the surface and gradually narrowing with depth, presumably becoming a fissure extending all the way to the upper mantle where the pressure and temperature are suitable for the formation of diamonds (45 kbars and 1000°C approx.). Why are they so deep? How were they formed? Moreover, if they are volcanic in origin, as they appear to be, why is no lava associated with them? The only plausible conclusion is that the pipes were caused by extremely violent eruptions of gas that blasted a hole through 150 km of overlying dense rock. The observation, that the pore spaces of natural diamonds contain highly compressed gases including CO₂ and CH₄ (ref. 19) and the result that heavy hydrocarbons clearly distinguishable from the surrounding rocks exist in the East Siberian pipes²⁰, including the observation that bore holes into these pipes yield significant quantities of CH₄ (ref. 21) enforce the following conclusions:

- (i) Unoxidised carbon exists in the outer mantle in the form of methane and hydrocarbons, as does pure carbon.
- (ii) Volatile-rich regions exist in the inhomogeneous mantle which have been giving off hydrocarbon gases long after the formation of the earth's crust which can build up such great pressures that they simply crack through the crust in violent explosions.

Earthquakes. The eruptions of gas mentioned above, the generation of which is not disputed by the organic theory, should cause earthquakes. In fact, earthquakes have been observed to be associated with gas ejection throughout recorded history.

Greco-Roman civilization. Anaxagoras first proposed the theory that gases ('air') were the cause of earthquakes⁵ (p. 49). Seneca, Pliny, Pausanias and Aelian mention the evolution of 'wind', strange animal behaviour, great flames rising from the ground, loud roaring noises, foul smell and peculiar fog and the development of peculiar odour and muddy appearance in the water of wells and springs occurring several days or months prior to the earthquake.

Anglo-Saxon age. Newton wrote that he felt 'sulphurous fumes' were the cause of earthquakes⁵ (p. 51). Mitchell reasoned that gases caused the slow, visible oceanlike waves that roll across landscapes during major earthquakes. He also points out that the sudden deaths of large numbers of fish would be most naturally explained by the evolution of poisonous gases from vents in the ocean floor. Alexander von Humboldt summarized the then accepted theory as 'elastic fluids seeking an outlet to diffuse themselves into the atmosphere' being the cause of earthquakes. Thousands of fish, many of a nature previously unknown to local fishermen, were found floating on the water in Monterrey Bay on the day of the destructive San Francisco earthquake of 18

April 1906 (ref. 5, p. 63). Similar reports come from Japan. Hydrogen sulphide, highly toxic to fish, is a likely candidate, killing the bottom dwelling fish that are not normally caught.

Chinese civilization. More recently, at the Sungpan-Pingwu earthquake (Aug. 1976), outbursts of natural gas from rock fissures were reported. Moreover, these sometimes ignited, creating fireballs. A total of 1000 were sighted. A few hours before the earthquake, the water in local wells was observed to exhibit a violent bubbling⁵ (p. 61, 62).

These reports spanning recorded history show that methane gas is closely associated with earthquakes. In fact it is not unreasonable to suggest that earthquakes are caused by enormous build-ups of highly compressed gases containing mostly methane. This is in fact a strong support in favour of the non-organic theory, since the amounts of methane evolved are too large to have been produced by biological sources.

Mud volcanoes. These instead of ejecting lava and gas like ordinary lava volcanoes, emit mud and gas instead. The cones built up by them, consisting of solidified mud, are similar to, but smaller than, those built by the lava volcanoes. They emit mostly methane, while smaller amounts of other hydrocarbons are also present, including other inorganic gases like He, H₂, CO₂ or steam. Many mud volcanoes simply eject high pressured unconsolidated mud. In contrast, lava volcanoes emit mostly carbon dioxide and water. Mud volcanoes closely follow the underlying fault lines. This is not just commonplace, but holds for all mud volcano regions of the world. Moreover, the quantities of gas required to produce the Soviet mud volcano fields have been estimated to be several times the total gas content of the largest known gas field⁵ (p. 101). How does one explain this?

The conventional explanation in terms of the organic framework is that the gas is generated by bacterial action on the organic content of the mud. However, this has some problems:

- (i) The gas so generated would bubble up on a continuous basis, and hence extremely violent explosions of the type observed in the major mud volcano regions of the world would be extremely unlikely, as large concentrations of gas most probably not build up.
- (ii) Chemical analysis reveals that the methane also contains significant amounts of methane, propane and other hydrocarbons. Moreover, mercury, helium and other trace elements occur in the gases. The carbon isotope ratio is sometimes quite different from that expected to be obtained from a biologically derived material.

The inorganic explanation is that the gradually upward migrating gaseous hydrocarbons build up beneath impervious rocks, and then after having built up sufficient

pressure, smash through the overlying rock, creating violent explosions of the type observed. These violent displacements of gas will cause violent turbulence of the water, which would stir up the fine-grained sediment, creating the mud so characteristic of these volcanoes.

Pockmark-like craters on the ocean floor. Crater-like markings on the ocean floor have been reported from the Adriatic, the North Sea, the Gulf of Mexico, the Orinoco Delta, the South China Sea, the Baltic, the Aegean, near New Zealand, and off Nova Scotia²². Sonar experiments in the North Sea reveal shallow, circular ridges ranging from a few metres to 200 metres in diameter over an area of 20,000 square kilometres, roughly coinciding with the oil and gas producing region. It appears that individual events were responsible for creating large fields of these 'pockmarks', since one set of pockmarks occurs 10 m below an overburden of more recent sediment, while the other is visible on the surface. Hence it is estimated that while one such event occurred within the last thousand years, another occurred 10,000 years ago. Since small trickles of gas produce small steep-sided cones of mud (as in the Gulf of Mexico, where bubbles issue from the top of these miniature volcanoes), sudden releases of gas must be responsible for the craters. Primordial gas is a good candidate to explain the pockmarks.

The author also points out a remarkable coincidence between the major mud volcano regions of the world and the major oil-producing areas: (i) The Persian Gulf, (ii) the Caspian, (iii) Indonesia and (iv) Venezuela.

The South Alaskan mud volcanoes emit mostly carbon dioxide and are situated near lava volcanoes. Only three mud volcano regions are not correlated with any oil-producing regions: S. Italy, New Zealand, Black Sea. This connection arises naturally in the non-organic approach, since mud volcanoes indicate cool regions of hydrocarbon migration.

Extra-terrestrial hydrocarbons

Meteorites. If primordial hydrocarbons were incorporated in the earth during the process of formation, then one should expect to find such substances in ancient material dating from the formation of the solar system. Such material exists in the form of carbonaceous chondrites, a class of meteorites. Moreover, this type of meteorite seems to be very common. In fact, asteroids and inter-planetary material seem to be of largely carbonaceous derivation²³.

Materials previously thought to be exclusively biological in origin have now been found on meteorites. Porphyrin-type molecules are found in meteorites and are almost certainly not of a biological derivation³.

Planets. The outer planets have their atmospheres largely in the form of hydrocarbons, chiefly methane. Uranus' atmosphere may contain as much as 14% of methane gas²⁴ (p. 221). Neptune's atmosphere consists of hydrogen, helium and methane while the inner liquid shell is thought to consist of water, methane and ammonia²⁴ (p. 233).

Comets. Halley's comet (1986) was found to emit hydrocarbon gases. The core was observed to be black, presumably because of it being composed of carbonaceous material. Lang and Whitney describe the interior as blacker than coal, its blackness perhaps being due to 'an admixture of minerals, organic compounds and metals'²⁴ (p. 254).

Isotope and trace element anomalies. The following peculiarities point to an extensive upward migration of deep fluids. Moreover, mantle-derived material occurs in association with petroleum:

Helium. This is closely associated with petroliferous regions. In fact the world obtains its commercial supply of helium by separation from natural gas¹¹ (p. 69).

Argon-40. Argon and its isotope Ar-40 occur in extraordinarily high concentrations in gas fields¹¹ (p. 70). Moreover, assuming the source of the 0.1% Ar by volume in the huge Panhandle gas field to be the source rock itself implies that the source rock must have been 100% potassium to supply the required levels of Ar-40 (ref. 25). Moreover, high values of Ar-40/Ar-36 are taken as an indication of mantle-derived material²⁶ (p. 417), strikingly petroleum displays this signature.

Experimental verification

The final proof would involve an actual experimental verification of the theory. Deep wells are good tests, since organic materials cannot occur in crystalline basement rocks. Several are under way:

The Kola superdeep hole. At 12 km, this is the world's deepest well (1984). Located in the Kola peninsula, now Russia, it reached deep down into the crystalline basement. The drilling released flows of gas at all levels. The liberated gases included²⁷ helium, hydrogen, nitrogen, methane and other hydrocarbons and CO₂. This provides convincing support for the suggestion that hydrocarbon gases exist at such great depths inside the earth that they cannot be of a biological origin.

The German deep hole. This hole is located in Windischeschenbach (Oberpfalz). The depth reached during the pilot drilling programme was 4 km (1990). From 3.2 km down the drill encountered increasingly common cases of highly concentrated salt brines with gases like

methane and helium in open caves in the rock¹⁶, but no petroleum.

The Swedish hole. The discovery of an oil and/or gas field in a location ruled out by the organic theory would settle the matter once and for all. Hence, Gold⁵ (p. 172) after studying various formations across the world, concluded that the Siljan Ring, Sweden was the best candidate for the job. This is the largest impact crater in Europe. According to the non-organic theory, the impact could have led to the formation of sizeable hydrocarbon deposits since the fractures created by the impact would favour the upward migration of primordial hydrocarbons. Although the field was located primarily on the basis of seismic data, numerous oil seeps have been noted in the small sedimentary deposits of the ring-shaped depression marking the crater, carbonates characteristic of oxidised methane occur in the area and seismic observations reveal zones of porosity stacked on top of one another. Hence the primary indications were favourable.

Finally, Gold succeeded in convincing investors and a project began to prospect for petroleum in the area. This was largely supported by the state-owned Swedish electricity utility Vattenfall. Drilling began in 1986. By late 1987 a depth of 6.5 km had been attained, but no large commercial deposits had been discovered²⁸. Opponents saw in this the death knell of the non-organic theory (claiming the hydrocarbons detected were from the lubricating drilling mud injected into the ground during drilling), while Gold proclaimed a victory (claiming that significant amounts of hydrocarbons were discovered, and that large amounts lay beneath)²⁹. Due to drilling difficulties, the project stopped short of its target. It can be said that this did not rule in favour of either proposition.

However, in 1989 the Swedish drilling company Dala Djupgas Produktions recovered a small quantity of oil from 6.7 km below the Siljan Ring. Again critics dismissed the find as being recycled drilling fluid. The tables were turned yet again when the same Company discovered oil in 1991 even at the shallow depth of 2.8 km at a nearby well, the horizon of the petroleum being basement granite³⁰. Moreover the previous objection was nullified as the drilling fluid used in this case was water only. The proponents of the organic theory claimed that this oil was merely oil that had seeped into underlying fissures in the basement of rock from oil shales, since the petroleum found in the oil shales and that in the basement rock were chemically very similar. However, the non-organic theory explains this as being due to the upward migration of primordial petroleum; the two oils are similar because their common source is the same. The upward migrating hydrocarbons would have produced both the deep oil and the oil shales, the

shale providing a good trap rock that could absorb the oil on its way up. Hence, the case appears to have recently swung in favour of the non-organists.

Only drilling in the future by men of the calibre of Col. Drake (the discoverer of the world's first oil-field), Dad Joiner (the discoverer of the giant E. Texas oil-field) and P. Higgins (the discoverer of the Spindletop oil-field) can yield the answer to this intriguing question.

Conclusion

The positive and negative features of the classical organic theory have been discussed. This has been the traditionally accepted proposal, much work having been done in this field. The rival non-organic theory has so far not been accepted due to the successes of the biological theory to date in elucidating certain properties of oil-fields. However, new results from deep holes across the world are difficult to describe in terms of the biological theory. It has been shown that these new observations can be naturally explained within the non-organic framework, and that the older biological supports (mainly relating to the presence of supposedly biological material in petroleum) can also be incorporated. Hence a duplex theory combining features of both theories may be the final victor. This would perhaps involve the enrichment of existing organic hydrocarbon deposits through non-organic hydrocarbons.

The abiogenic theory derives much of its support from diverse and exotic fields such as astrophysics, cosmo-geophysics, thermodynamics, nuclear geology, etc., and considerable strides in the comprehension of these fields have led to a impressive growth of information in support of the non-organic theory.

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MEETINGS/SYMPOSIA/SEMINARS

National Seminar on Bamboos and Exhibition of Bamboo Crafts

Date: 28-29 November 1996
Place: Bangalore

Seminar on status of bamboos in India and an exhibition and sale of bamboo products are arranged.

Contact: Dr H. M. Swaminath
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Malleswaram,
18th Cross
Bangalore 560 003
Phone: 3311682
Fax: 080-3362935

National Symposium on Power System Instrumentation and Control

Date: 26-27 December 1996
Place: Bangalore

Topics include: Power electronics/power quality, Diagnostics and expert systems, Communications, DA/DSM, Grid operation/load despatch, Standards and standardization, Action plans, Thermal power plants, Protection, Optimization, Simulation and problem oriented papers.

Contact: Shri R. K. Hegde
Joint Director
Instrumentation Division
Central Research and Testing Laboratory
Central Power Research Institute
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Bangalore 560 094
Phone: 3412915, Fax: 080-3416213

National Conference on Alternative and Renewable Energy Technologies

Date: 10-11 January 1997
Place: Hyderabad

Topics include: Solar thermal applications, Solar photovoltaic technology, Wind energy generation, Hybrid energy systems, Biomass conversion, Energy conservation and audit, energy recovery and efficient systems, Natural gas applications: fuel cell, cogeneration, combined cycle power plants, Hydrogen energy, Alternative fuels and Ocean energy technologies.

Contact: Prof. D. N. Reddy
Organizing Secretary
Department of Mechanical Engineering
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Hyderabad 500 007
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National Seminar on Coastal Zone Environmental Management: An Appraisal of the Contemporary Research and Development

Date: 12-14 February 1997
Place: Mangalore

Themes include: 1. Coastline and coastal zone (material and processes), 2. Remote sensing and coastal zone studies, 3. Coastal zone ecosystems, 4. Industrialization and coastal zone environment (impact of infrastructural development, waste disposal, etc.), 5. Water resources of coastal zone, 6. Coastal regulation zone (CRZ) (delimitation of CRZ, level of implementation of CRZ Act).

Contact: Prof. T. R. Sreedhara Murthy
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Sálim Ali

(12 November 1896 – 20 June 1987)

Madhav Gadgil

Sálim Moizuddin Abdul Ali was unquestionably the greatest whole organism biologist of 20th Century India. His insightful observations of bird life straddled most of the century, for the earliest of these were recorded in 1907 when he was all of eleven years old. They read: 'The cock sparrow perched on the nail near the entrance to the hole while female sat inside on the eggs. I ambushed them from behind a stabled carriage and shot the male. In a very short while the female acquired another male who also sat 'on guard' on the nail outside. In next 7 days I shot 8 male sparrows from this perch. Each time the female seemed to have another male in waiting who immediately stepped into the gap of the deceased husband.'

Sálim Ali learnt about birds, not from books, nor from musty specimens in museums, but through practice; by watching them at first hand, and in days before anybody thought of conservation, by ambushing them, and shooting them for the pot. It was just such a sparrow he shot the next year, when he was twelve, that turned out to be a different species, a yellow throated sparrow (*Petronia xanthocollis*), which brought him in contact with Bombay Natural History Society, its collection of plant and animal specimens and books on natural history. From that time on he was hooked on a biological career; a career he pursued with such distinction, with little formal training. For in his first year in the college he stumbled on 'logarithms and such like

evils' and ran away to Myanmar to work with a cousin in mining wolfram. Deciding, however, that he was not cut out for business, he returned to Mumbai, read for a B A in Zoology at St. Xavier's and later spent a year of practical training in taxonomy at the Berlin University Zoological Museum. For most of his life, he held no jobs, but pursued ornithology at the Bombay Natural History Society¹.

As the philosopher Wittgenstein remarks 'practices reveal meanings'. This injunction is particularly germane for organismic biology, for the study of complex phenomena that students of this discipline confront admits of no universal generalizations. Ecology, ethology, biogeography, systematics, can therefore only advance on the basis of practical, precise knowledge about particular plants, animals, microbes or habitats². Sálim Ali spent a life time collecting such practical, precise knowledge on many aspects of Indian bird life. He unravelled the fascinating breeding system of Weaver Birds and the role of sunbirds and flowerpeckers in pollinating and dispersing seeds of mistletoes. He undertook a series of regional bird surveys of Hyderabad, Travancore-Cochin, Afghanistan, Kailas Manasarovar, Kutch, Mysore, Sikkim, Bhutan, Arunachal Pradesh. He investigated the famed Flamingo city of the Rann of Kutch, and ringed wintering water-fowl at Bharatpur to trace their migratory routes.



A. R. Rahmani

Throughout he maintained the most meticulous records and distilled his knowledge into a series of superbly written and illustrated books; beginning in 1941 with the *Book of Indian Birds*, followed by the *Birds of Kutch*, *Indian Hill Birds*, *Birds of Kerala*, *Birds of Sikkim*, and culminating in his magnum opus, the ten volume *Handbook of Birds of India and Pakistan*. His last book, the *Field Guide to the Birds of Eastern Himalayas* was published in 1977. It is these books that have been sold many times over and will undoubtedly continue to be printed and reprinted for decades to come, that have instilled a love of natural history in a section of India's educated classes, who have otherwise been singularly insensitive to the charms of tropical nature that surrounds them. For the books are not only scientifically accurate; they make for immensely pleasurable reading; Sálim Ali was not only a great naturalist, he was a man of sparkling wit and a master of the English

language as well.

Sálim Ali was a true aristocrat, a scion of the famous Badurddin Tyabji family, a personal friend of Jawaharlal Nehru and Indira Gandhi, a Padma Bhushan, a Member of Rajya Sabha, a Fellow of many scientific Academies and a winner of numerous prizes in science and conservation. But above all, he will be remembered as the man who taught Indians to appreciate, to study at first hand, to treasure, to work towards conserving the rich living heritage of the country.

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Sálim Ali — A tribute*

J. C. Daniel

Way back in 1951, after a hard day of field collecting for Dr Sálim Ali's ornithological survey of the Berars, I was relaxing with him in the forest rest house at Chikalda, now part of the Tiger Project Sanctuary of Melghat in Maharashtra. I had joined the Bombay Natural History Society the previous October, and I had been brought out on a bird survey and was very much on trial. Apparently I had not been found wanting. The 'old man', a term which was used with the greatest respect by the staff of the Society who were very fond of him but who found his unbending principles rather trying occasionally, had decided to unbend a little and we were discussing my background.

When he realized that I had been brought up in Kerala, he talked about the bird survey he had conducted in Kerala, then the States of Travancore and Cochin, in the 1930s and the people he had met. One among them was a Dr Jivanayakam who had been secretary of a fact finding committee, which was investigating the practices, both good and bad, of aided educational institutions. The Committee and Sálim Ali's survey party often shared the same dak bungalow and they had become friends particularly when the old man found that the other had more than a casual interest in the study of birds, though his specialization was in education.

As we talked about this person he asked me whether I knew or had heard about him. When I told him that Dr Jivanayakam was my father he was struck dumb with amazement. As he described this incident in his autobiography, *The Fall of a Sparrow*, it was one in a million chance that he should have been working with the son of a man he had known decades earlier. I think neither my father nor I had tried to contact him or speak of this acquaintance when I was trying for the job of research assistant at the Society. The rapport that we then struck stayed with us for the next 36 years of our association. To me, as to the many scientists who joined the Society during this period of our association, he was a father figure to be emulated, for there was little that was not good in him. His attitude towards work, for instance. He was a person who believed in striving hard when opportunities offered the chance.

In the field we had no work hours but neither had he. If we worked 14 hours it was with the knowledge that he would certainly put in 18 hours and would not be paid a penny in the bargain. He believed, as Gandhiji did, in the dignity of labour and nothing was below his dignity to handle. He agreed with Gandhiji that it is not the type of labour that gives you status and dignity but that dignity rests on your own self-assessment and self-confidence.

A great and admirable lesson that one learned working with Sálim Ali is the gravity and care necessary in the handling of money, especially public funds. The accountability not only to the donor but also to oneself,

J. C. Daniel, former Director, BNHS is currently a member of the Executive Committee, BNHS and chairman of the Salim Ali Wild Wings Trust.

* Reproduced with permission from *Hornbill*, BNHS.

that it must be frugally spent and made to give the maximum benefit, was imprinted firmly in the minds of all his scientists and students. Nothing was said but the example was set. He had worked on shoe-string budgets throughout the major portion of his bird survey collection career, depending largely on the munificence of the now vanished species, the maharajas and princes of India. It was in their States that he did his major bird study surveys. The only assistance he had was from the BNHS which gave him the services of a Skinner for the bird collection that he gave them. I do not think that except for the time he was the nature education organizer in 1927, he ever drew a salary in his life till he became a National Professor of Ornithology in the 1980s. From the grants he received from the maharajas he drew nothing but his living expenses, all the rest was ploughed back into collection and study of his first love – the birds of the Indian subcontinent.

All the bird surveys had a target. In our Berar survey, we were specially looking for the white-fronted tree pie, a species which is now restricted to the rainforests of Kerala, but had once been recorded by a reliable ornithologist from Berar. The surveys along the Western Ghats, hills of central India, Bastar and Orissa looked at the discontinuous distribution of rainforest birds now restricted to the Western Ghats of the south and the forests of eastern India. He was collecting evidence for a hypothesis known as the Satpura hypothesis propounded by Dr Hora, the then director of the Zoological Survey of India, on the route of movement of these species now existing at two corners of an India, divided by over 2000 km of unsuitable country in between. The Hyderabad survey looked for the Jerdon's courser, a bird that was subsequently to be rediscovered in Cuddapah district by one of Sálím Ali's young field biologists. Another notable rediscovery was of Finn's baya after a period of over 50 years in the Kumaon Terai.

Sálím Ali was as active in the field of conservation as he was in ornithology. He was probably the person who had travelled to all the obscure regions of the Indian subcontinent at one time or other of his life and knew the country and its forest intimately. His knowledge and experience were respected, and his timely intervention saved, for instance, the Bharatpur Bird Sanctuary, now the Keoladeo National Park, and the Silent Valley National Park.

As a prelude to the magnificent ten volumes on Indian birds which he completed by 1974, almost all the surveys gave rise to a book, *The Birds of Kutch, The Birds of Travancore and Cochin*, later published as *The Birds of Kerala, The Birds of Sikkim and The Birds of the Eastern Himalayas*, each a popular version of the data collected by the surveys of the area. These and the

ever popular *Book of Indian Birds* were to familiarize bird watching and bird study as excellent forms of relaxation in a stress-filled world.

His surveys and individual bird studies were examples of how much information can be obtained with a minimum of equipment, a notebook and pencil, a pair of binoculars and an alert, analytical mind. The precise notes he made during his bird surveys remain some of the best examples of data collecting.

It was a teacher that Sálím Ali really excelled. The Bombay University had recognized the Society as a Research Institute in Ornithology with Dr Sálím Ali as the research guide. His methods were innovative and the student was left to develop his own ability and initiative, with guidance subtly rendered through discussions. The bond that was so established, was in the best traditions of the Indian *guru* and *shishya* relationship. He was thus able to expand the research capabilities of the Bombay Natural History Society when the opportunity offered.

Recognition came late to Sálím Ali but came abundantly. The Asiatic Society's gold medal for researchers in Asiatic Zoology, Padma Bhushan and late the Padma Vibhushan for continued distinction in zoology from the Government of India, the Sunderlal Hora Memorial award of the Indian National Academy of Sciences for 'outstanding contributions to Indian ornithology'; The degree of D Sc from the Universities of Aligarh, Delhi and Andhra. The Union Gold Medal of the British Ornithologists Union and several other international awards of recognition including the Paul Getty International Prize for Wildlife Conservation.

The BNHS was very much a part of Sálím Ali's life from the time he timidly entered its portals as a small boy with a yellow throated sparrow in his hand. He was a member of the Society for over 69 years and the organization gradually became synonymous with him. It was his family and all that he cared for. To the Society he left whatever he thought was valuable in his possession.

A man with a fine natural modesty, he was humane, selfless, sensible, and with a lively sense of humour. Above all, he had what Gandhiji also had and which the Arabs call 'Baraka', the quality of being able to bestow blessing or benediction.

Sálím Ali was a non-conformist, a man who for many years walked a lonely path divergent from the main stream of science in India. It is a tribute to his determination and genius that at the end of his life he had a sizeable population of the conformist main stream following him, or at least appreciating and commending his more or less single-handed efforts to present the study of the birds of his land, the ethereal spirits of the air, to his countrymen and to the world.

Deploying student power to monitor India's lifescape

Madhav Gadgil

Along with his many scientific contributions, Sálim Ali will be remembered for a whole series of superbly written and illustrated books on Indian birds, books that played a key role in stimulating popular interest in India's rich living heritage. In honour of this great naturalist the Indian Academy of Sciences will launch on the occasion of his birth centenary a project called 'Lifescape' as a part of its initiative to enhance the quality of science education. This project aims to publish illustrated accounts of 2500 to 5000 Indian species of microorganisms, plants and animals. These accounts would help high school, college and postgraduate students and teachers of biology to reliably identify these species, and thereby constitute a basis for field exercises and projects focusing on first hand observations of living organisms. The information thus generated could feed into a countrywide system of monitoring ongoing changes in India's lifescape to support efforts at conservation of biological diversity, as well as control of weeds, pests, vectors and diseases. These accounts would also help create popular interest in the broader spectrum of India's biological wealth, much as Sálim Ali's books have done for birdlife over the last fifty years.

INDIA is a land of great natural diversity, diversity that embraces mangrove swamps of Sunderbans and rain forests of Western Ghats, coral reefs of Lakshadweep and wetlands of Bharatpur, hot deserts of Rajasthan and cold ones of Ladakh. Thanks to this diversity of environmental regimes and its position at the trijunction of African, Eurasian and Oriental biotas, India is one of the world's 12 megadiversity countries. Over 125,000 species of living organisms have been described from our subcontinent; it probably harbours another 400,000 awaiting to be described. India is also one of the global centres of diversity of crops and livestock¹⁻⁵.

This biodiversity and its knowledge is now assuming great significance, for the second half of twentieth century belongs to sciences and technologies of life. Beginning with the elucidation of the chemical nature of heredity, life sciences have made rapid strides in understanding of the working of the machinery of life. Knowledge is power; and this understanding has been translated into an ever growing sophistication in manipulating living organisms. This has opened up many novel possibilities of application, and it is expected that the resulting biotechnologies may account for as much as 40% of the world economy in the coming decades. The stupendous diversity of life is the raw material for these applications and an understanding of this diversity must go hand in hand with a deepening understanding of the working of life to reap fully the fruits of modern advances in biological sciences^{6,7}.

Our weaknesses

Unfortunately, India's scientific base of knowledge of this diversity of life and ways of adding value to it is very weak. Only some 20% of the species we harbour are likely to have been scientifically described; but even of these a large proportion has been described by British and other Western scientists⁸. Their specimens are located in the Museum of Natural History or Kew Herbarium in London; but are often absent from Indian collections. Thus A. K. Ghosh (pers. comm.), formerly Director of Zoological Survey of India reports that of 82,000 described species of animals, specimens of only 51,000 species are located in the Indian Museum in Calcutta. Specimens of an additional 1000 animal species may be available with Universities or institutions like the Bombay Natural History Society; that still leaves 30,000 species for which the specimens are available only abroad. For some groups like birds we have in India specimens of all the species; but, even in this case there are likely to be 10 times as many specimens abroad as in India (J. C. Daniel, pers. comm.). On the other hand, with animal groups like aphids, India holds specimens of only about 10% of the described species (A. K. Ghosh, pers. comm.). Aphids are important crop pests and now foreign agencies holding Indian material are charging thousands of rupees for help in identifying a single specimen. We are also very unfavourably placed with respect to crop genetic resources the rice germplasm collection of the International Rice Research Institute is, for instance, richer, even in terms of Indian material, than any of our own collections. We do not have a single properly organized and internationally

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recognized repository of microbial cultures either.

We are equally unfavourably placed in terms of scientific capabilities of identifying, working with, adding value to biodiversity resources. India boasts of the third largest scientific manpower in the world. Our Botanical and Zoological Surveys have very long history, and together employ over 1000 trained biologists. Our more than 6000 colleges and University departments employ another 20,000 or so. Every year more than 100,000 students get a bachelor's degree in one of the branches of Life Sciences. But only a very small fraction of these get an exposure to India's living wealth. Practically none of our master's degree holders in zoology is able to name more than 5 to 10 species of birds, lizards, fish or butterflies put together. This is because they are never encouraged to look at living creatures abounding around them, their training being confined to identifying a few dead specimens, or dissecting a still living cockroach or a dead pigeon. Given such a training programme few teachers of biology know much of the living wealth of India either.

Monitoring biological populations

Identification of the great diversity of living organisms around us, a majority of whom, especially smaller animals like mites and marine animals like bottom dwelling worms are yet to be described is a challenging task. This would call for a high level of professional training and infrastructure. But equally significant is a task which can be undertaken without sophisticated technical inputs – this is the task of monitoring the ongoing changes in the populations of living organisms through the length and breadth of the country, a task we are committed to undertake as a party to the Convention on Biological Diversity^{8,9}. It is very much in our interest to do so for a variety of reasons – to appreciate if populations of medicinal herbs are on decline through overharvests, or if wild relatives of cultivated plants are being eroded through habitat transformations; to appreciate if populations of weedy species such as water hyacinth are exploding and choking our wetlands, or whether new species of insects are assuming pest status for our crops; and to keep track of populations of vectors of human diseases such as mosquitoes. This is a task which does not call for a capability to identify all of our known 125,000 species – or unknown 400,000; but a task which can be effectively discharged by developing the capability to reliably identify a few hundreds, or a few thousands of species. The Foundation for Revitalization of Local Health Traditions has identified a set of 300 species of medicinal plants of particular significance from Karnataka, Tamil Nadu and Kerala. To this we may add a couple of hundred species of edible fishes, a

few hundred species of butterflies, frogs, turtles, birds, mammals of conservation significance, a few hundred species of insect pests of crops, a few hundred species of bacteria, fungi, intestinal worms and other human and livestock parasites and their vectors and so on. The list may come to a few thousand species of considerable significance that ought to be monitored throughout the entire stretch of our country's landscape, waterscape and seascape. Only a few hundred out of this set of a few thousand may be present in any given locality. To develop the capability of reliably identifying such a subset is not a difficult task at all; it is a task that could be mastered by any interested lay person. After all many enthusiastic bird watchers can readily identify two hundred to three hundred species of birds; and every practising fisherman and woman easily identifies a few hundred species of fish and other marine animals. It is therefore quite possible for students and teachers of biology to acquire such a capability, each for their own locality, given adequate support in terms of literature, and given that there is adequate motivation to acquire this knowledge.

Two facets of biology

To assess this proposition, we must take a second look at the teaching of biology. Teaching and research in biology are addressed to two facets of life:

- (a) Functioning of life based on a machinery largely held in common by organisms as different as bacilli, moulds, trees and birds,
- (b) The resulting diversity of living organisms and their associations.

The functioning of life is the subject matter of the disciplines of biochemistry and molecular biology, physiology, developmental biology and genetics. The diversity of life is the subject matter of disciplines of morphology, taxonomy, evolution, ecology, ethology and biogeography. The former set of disciplines may be termed functional biology, the latter organismic biology. The teaching of these two branches, functional biology and organismic biology poses different challenges. In teaching functional biology the emphasis has to be on the underlying commonalties; genetic code is genetic code, and Krebs's cycle or transmission of nervous impulses across synapses are the same whether taught in Madurai or Madrid, Malawi or Massachusetts. In teaching of organismic biology, however, the focus can, with profit, be very different. The variety of birds or butterflies is very different and much greater in Madurai than in Madrid, in Malawi than in Massachusetts. To teach functional biology in Madurai on the basis of textbooks and laboratory experiments designed in

Massachusetts can be very sensible, to teach organismic biology in a similar fashion does little justice to the wealth of life in and around Madurai.

There is yet another significant difference. Our understanding of functional biology is rapidly advancing. But this calls for probing into the machinery of life with sophisticated instruments, with expensive chemicals. Given our limited financial resources, Indian universities and colleges are at a disadvantage in arranging laboratory exercises for teaching many aspects of functional biology. Our understanding of organismic biology is also advancing, though not at the same rate as our understanding of functional biology. Twentyfive years ago, it was estimated that there were around 3 million different species of living organisms, of which some 1.6 million had been described. Subsequent studies have led to an upward revision of this estimate to somewhere between ten and thirty million. This implies that most of the diversity of life has not even been documented in terms of a simple description and naming of species, let alone taken further in understanding of the ecological role, geographic distribution, evolutionary relationships of that species. Most of these unknown species are believed to occur in tropical forest and coastal habitats. They must be identified, their distribution, their ecological role investigated where they occur in their natural setting; that means a tremendous opportunity for us in India in adding to the store of biological knowledge.

Untapped opportunities

Of course, some of these investigations require considerable support in terms of literature and museum or herbarium specimens^{8,10}. Tragically little of such support is available within our country. But description of new taxa is only one part of research in organismic biology; information on the distribution and abundance of many species of known significance is also of considerable relevance. As stressed above, much of this is of applied value – we need to know whether populations of medicinal plants like sarpagandhi, *Rauwolfia serpentina* or wild relatives of cultivated plants like wild rice, *Oryza nivara*, are threatened with extinction, where and when populations of crop pests like brown leaf hopper or vectors like anopheline mosquitoes are undergoing rapid increase. Such information is vital to efforts at conservation of our rich biodiversity resources, and attempts at control of pests, diseases, vectors.

This kind of information, information for monitoring populations of thousands of species of human significance needs to be continually collected from all over the country. There are of course centralized agencies entrusted with the task of doing so, but they are unable to do full justice to this responsibility. Thus we do have a

Botanical Survey, a Forest Survey, a Central Institute of Medicinal and Aromatic Plants; yet we have essentially zero information on the status of hundreds of species of medicinal plants of the country. We do have a Central Institute of Communicable Diseases, but its machinery could not monitor the outbreak of rat and flea populations preceding the plague outbreak of 1994.

Obviously these centralized organizations must be supported by a much more widespread network of centers for monitoring the populations of a number of species of living organisms across the country. Undergraduate science colleges along with high schools and centres of post-graduate studies are clearly the answer. These span every one of country's 500 districts, a vast source of scientific competence that has been made little use of. The botany, zoology, microbiology teachers and students of these colleges could easily study and document the distribution, abundance and seasonal and annual changes in populations of a few hundred species of organisms in their own localities. Indeed, there have already been several interesting experiments along these lines. Around the Palamau Tiger Reserve in Bihar, biology teachers from some undergraduate colleges have formed a network for monitoring the Tiger Reserve ecosystem working in collaboration with the Wildlife Wing of the State Forest Department (D. S. Srivastava, pers. comm.). The Ahmedabad based Center for Environmental Education has extensive programmes of involving high school and college teachers in monitoring a variety of environmental parameters¹¹. The Western Ghats Biodiversity Network coordinated through the Centre for Ecological Sciences at the Indian Institute of Science involves botany and zoology teachers from about 15 colleges working with teams of 5–15 students to map local landscapes and investigate the occurrence of species of a number of groups of plants and animals including mosses, flowering plants, aquatic insects, fishes and birds¹². Such studies not only would constitute a valuable learning experience, they would also generate considerable information of potentially applied value which could feed into a properly organized bioresource monitoring system of the country.

Fresh approach

It is thus entirely possible to take up this challenge. But to do so successfully calls for a fresh and different approach to the teaching of organismic, in contrast to functional biology. Functional biology has to be taught on the basis of a largely uniform curriculum, and a curriculum focused on laboratory experiments. Organismic biology could be taught much more meaningfully on the basis of a flexible curriculum focusing on locally occurring plants, animals and microbes, and should supplement laboratory exercises by extensive field observations. These requirements of flexibility, of scheduling

field observations, and of assessing field based studies do pose difficulties, but these are challenges that can surely be surmounted with some effort.

Such an approach need not however involve additional burden of course work for students and teachers. Our high school curricula already include environmental studies. Our B Sc and M Sc biology courses provide for teaching of morphology, classification, evolution, ecology, biogeography. What needs to be done is that part of this material be substituted by other material – more flexible, and emphasizing first hand observations. Thus in zoology courses we have a paper on chordates. As a part of this paper students learn of platyrrhines, the primitive monkeys found in South America, but they do not learn that Jodhpur has troops of hanuman langur, *Presbytis entellus* which has been the subject of fascinating ecological and behavioural studies in Rajasthan, Gujarat, Uttar Pradesh and Himachal Pradesh. They are not encouraged to estimate these monkey populations or observe their social behaviour. At the same time, the Government of India has spent huge sums of money to sponsor through Zoological Survey of India a survey of primate populations of India, a project that was abandoned half way through, a project whose results have never been published. If all zoology students in India had been taught of the identity of the macaques, langurs, gibbons in their respective localities and encouraged to yearly maintain records of the monkey troops in their own area, we could have accomplished not only a one time primate survey, but an ongoing monitoring of primate populations in a most cost effective fashion. It would be no serious loss if in the process, we teach the zoology students a little less of squirrel monkeys in South America.

Such broadbased programmes of monitoring populations of some selected set of species have been successfully conducted in many parts of the world, primarily drawing on the resources of amateur bird watchers. The best known of these is the Christmas bird count of the National Audubon Society in the United States of America. Started in 1900, the programme now involves over 45,000 volunteers. The counts are conducted in over 1500 designated areas, each 12 km in radius. Within a week or two of Christmas, each designated count area is censused for one day, with participants seeking out and counting all birds in the area¹³. Parallel exercises have been conducted in India, such as the wintering waterfowl count sponsored by the Asian Wetland Bureau. Project Lifescape visualizes similar countrywide monitoring, drawing on biology teachers and students and covering the entire spectrum of living diversity.

Lifescape of India

An interesting approach to teaching organismic biology

in this way would be to do what was proposed above, i.e. to come up with an overall list of plant, animal, microbial species of India whose first hand observations might form an important component of teaching of environmental sciences or biology – not all species everywhere, but in some part of India or other. For instance, this list may include a few of primate species – (1) bonnet macaque, (2) rhesus macaque, (3) Assamese macaque, (4) hanuman langur, (5) Nilgiri langur, (6) hoolock gibbon. Only one of these species may be selected for study in any given locality – for instance, bonnet macaque in Madurai, hanuman langur in Jodhpur, hoolock gibbon in Shillong and so on. This is not to suggest that students should close their eyes to species outside this list; the listed focal species should instead serve to build a foundation for getting students involved in observing other living organisms as well. Employing the study species as an example, the students would then observe its members under natural conditions and in the process become familiar with the morphological characteristics of primates, the place in classificatory scheme and evolutionary relationships of macaques as a part of the Chordate paper. They could observe their habitat use, food preferences and estimate their numbers as a part of the ecology or environmental biology paper, observe their social behaviour as a part of the behaviour paper, look at their geographical distribution as a part of the biogeography paper, and so on. Some of their observations could also feed into a country-wide project of monitoring primate populations.

The six primate species noted above would be part of an overall national list of microorganisms, plants and animals. This list has to be a subset of the estimated 500,000 and the described 125,000 or so species of India. We therefore need some criteria for inclusion in the total list. Possible candidates for such criteria include:

- Economic significance, for instance, medicinal plants, wild food plants, plants producing minor forest products such as tendu leaves, genetic resources such as wild relatives of cultivated plants or domesticated animals, animals which may be bred under domestication with profit, such as in butterfly farming, crop pests, weeds, crop pollinators, vectors of human diseases, fresh water fishes.
- Striking appearance and therefore ease of and attraction to observation: larger mammals, commoner species of birds, crocodiles, fireflies, dragonflies, plants with attractive flowers, large trees.
- Cultural significance: Monkeys, peafowl, *Ficus* species such as peepal.
- Conservation significance, for instance, endemic or threatened species.
- Desirability of having representatives available in all parts of the country, from Port Blair in Andamans to

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Leh in Ladakh, and from rainforests of Mizoram to concrete jungles of Mumbai.

- Desirability of having representation of all groups of living organisms. Thus we should include seaweeds, lichens, mushrooms, ferns along with higher plants amongst wild food plants.

How big should such a national list be? It has to be a subset of the 125,000 described species of living organisms. Any given educational institutions could readily include an area within a radius of 3 km; say a total area of 32 km² for the field studies. The total area of our country is 32 lakh km², larger by a factor of 10 to the power of 5. Ecological theory tells us that depending on the group of organisms concerned the total number of species encountered increases at a power of 0.2 to 0.4 with the area. This implies that a study area of 32 km² may harbour between 1% and 10% of the total; i.e. between 1250 and 12,500 species of microbes, plants and animals, all put together. These are likely to be overestimates, for the habitats readily accessible to educational institutions would surely be poorer in living diversity than the more natural habitats for which these estimates should hold. But even then students at any educational institution should easily have access to several hundred species of living organisms in their close vicinity. Anthropological studies in Amazonian forest have shown that indigeneous people have distinct names for as many as 1500 species of local plants and animals. Trained biology students could then also learn to know several hundreds, say 250 to 500 species without much difficulty. Following the rule of thumb of 1% to 10% of total set being present in the vicinity of any given school or college in the country, the national list has to lie in the range of 2,500 to 50,000. For reasons of practicality it is best to aim the lower part of this range; so we may aim for a national list of 2500 to 5000 species. Such a national list may be viewed as the list of indicator species for assessment of the status of biodiversity in the country for the purpose of Indian national reporting to the Convention of Biological Diversity^{9,14}.

It is our proposal then that the locally available subset of a few hundred out of this national list of 2500 to 5000 species be employed as subjects for first hand observation as a part of teaching of environmental science, or of branches of biology such as morphology, classification, evolution, ecology, biogeography, behaviour over the three years of high school, 2 years of junior college, 3 years of undergraduate and 2 years of M Sc curriculum. Again not every student may end up observing all the locally available species, many may be subject to special observation as an individual or group project by one or few of the more interested students. Table 1 suggests a possible taxonomic breakup of a set of 2500 species and Box 1 a possible way of apportioning these species at different stages of high school and

Table 1. Possible taxonomic break up of a set of 2500 species

Viruses	50
Bacteria	90
Algae	90
Fungi	200
Lichens	10
Bryophytes	10
Pteridophytes	10
Gymnosperms	10
Angiosperms	700
Protozoa	25
Sponges	5
Coelenterata	20
Platyhelminthes	10
Nematodes	20
Annelids	10
Minor phyla	10
Insects	700
Spiders	20
Millipedes, centipedes	10
Crustacea	25
Mites	25
Molluscs	40
Echinoderms	25
Fishes	120
Frogs	10
Reptiles	30
Birds	200
Mammals	25
	2500

college education. The Appendix provides a possible model account of one of these species including a set of possible student projects.

Environmental setting

This subset of focal species of any particular locality would occur in a specific environmental setting. It would be desirable that the curriculum include a first hand study of this setting which could become a part of the environmental sciences, biology, geography or geology paper. Indeed study of this setting is the subject of a newly emerging discipline known as landscape ecology. Landscape ecology views any region as a mosaic of repeated occurrences of a set of landscape element types which may occur as patches, for example, paddy field, grassland, pond, evergreen forest, scrub savanna, human habitation or as linear or branching elements such as watercourses, roads, electric lines. It would be desirable to select one particular area, say of ten to thirty square kilometers near the high school, college or University and to prepare a good map of the area in terms of occurrence of different types of landscape elements. Satellite imagery could be a very useful aid in the preparation of this map, and such a study programme could be linked to a programme of remote sensing literacy. This landscape map could then be the basis for organizing the

Box 1

The students of environmental science and of biology in any given locality may become acquainted with the subset of the total 2500 species present in their locality in a graded fashion beginning with the commonest, most striking, easily identifiable, most significant species and adding on the less common, less significant, more difficult species at higher standards. Consider as an example, 95 species of birds (out of a total country wide set of 200 species) present in any given locality. In the 8th standard the students may begin with Jungle Crow, Indian Myna, House Sparrow, Roseringed Parakeet and Pariah Kite, adding species like the Ashy Wren-Warbler, Blyth's Reed Warbler, or Sparrow Hawk at the 2nd or 3rd year B Sc level. The 95 species may be covered in the following sequence. VIII (5), IX (5), X (5), XI (10), XII (10), I BSc (20), II B Sc (20), III B Sc (20). There would of course be no expectation that the students would not explore and get to know bird species outside this list. In fact, the account for the Indian Myna would mention how to discriminate it from very similar bird species such as Jungle Myna drawing attention to this additional species. In a locality where Hill Mynas are present, the students would naturally notice them as well, once they begin observing Mynas even if the Hill Myna may not figure amongst the list of 200 species of India selected for the purpose of this programme.

study of distribution, abundance, behaviour of the focal subset of species. It could, for instance, be used to organize information on distribution of habitats where mynas or mosquitoes breed, or a certain species of medicinal plant occurs. Ancillary information, such as older satellite imagery, official documents or oral accounts by local people could be used to reconstruct the dynamics of change in the study locality and to use it to infer changes in availability of habitats preferred by species under investigation.

Economic significance

A study of the economic significance as well as the folk knowledge and usage of the focal subset of species could form a part of a paper such as environmental science/economic botany/economic zoology/applied biology/sociology/economics. The economic significance may range from a variety of services such as food or medicine or disservices such as crop pests or vectors of human diseases. Such studies could be linked to other parts of the curriculum as well. For instance, a local medicinal plant may be the source of a specific alkaloid which could be studied as a part of the biochemistry

course. The local uses of the medicinal plant could be studied as a part of the social studies, sociology or economics courses. In any event, linking the study of biology to species with specific known significance would greatly enhance the understanding of the subject by students, as well as motivate them to contribute to programmes of management of the species, whether of conservation or control.

Management regimes

A first hand study of ongoing attempts either at conservation or control of the focal subset of species, as well as of the land-use management of the entire study area at the landscape level could also be usefully incorporated in environmental science/biology curricula. Focusing on species level, there may be relevant local traditions such as protection to peafowl or peepal trees, or official measures such as a ban on hunting of all birds, or of cutting of any tree from a reserved forest. There may be programmes of propagation of medicinal plants, or control of rat or mosquito populations. It would be worthwhile studying the intentions behind such measures, as well as the efficacy of their actual operation in the field. It would also be worthwhile undertaking field studies and documentation of phenomena such as harvest, trade, marketing of medicinal plants or poaching of quails and partridges.

At the landscape level there would be a variety of regulatory measures, as well as developmental activities, both of which should be investigated and documented. These may include prohibition of encroachment on reserve forest land, or in the city prescription of the ratio of open to built up land. It would be worthwhile to monitor the efficacy of the regulatory measures, the ongoing changes and their impact on populations of the focal subset of species under study. Such investigations would not only be a useful device of teaching environmental science/biology but of generating an environmentally literate citizenry.

Organizing information

The exercises proposed above would be valuable to teaching of all aspects of environmental science/organismic biology. Additionally they could generate information of applied value in at least four different contexts.

(1) Conservation of valued species, such as wild relatives of cultivated plants, crop pollinators or threatened species of wildlife. Currently State Forest Departments, Forest Survey of India, Wildlife Institute of India, Botanical and Zoological Surveys of India are engaged in

some monitoring with this end in view.

(2) Utilization of economically valued species such as medicinal plants. Currently some drug companies have limited monitoring of their resource species.

(3) Surveillance of crop pests or livestock diseases. Currently state departments of Agriculture, Horticulture, Animal Husbandry and Veterinary Services have monitoring programmes of this nature.

(4) Surveillance of vectors of human diseases. Currently the National Institute of Communicable Diseases has limited programmes of such monitoring.

Potentially the teaching programme sketched above could generate much information of value in all these contexts. While all environmental science/biology students from 8th standard onward may be involved in the broader programme, a limited set of motivated students may participate in generating more reliable data to feed into an information system for applied purposes of conservation, economic utilization or control. Such students may be those who opt to undertake special projects as an optional part of the regular curriculum, or those who participate in some special activity such as a National Earthwatch Corps on the model of the National Cadet Corps or National Service Scheme.

This information should feed into new well-designed, vigorous programmes of monitoring all aspects of country's environment, including of course the landscape. Such a system should take full advantage of the modern informatics technologies and be organized as an interlinked network of computerized data bases^{1,7,15}. Educational institutions should be part of such a network involving a number of other agencies such as Forest, Botanical and Zoological Surveys, National Remote Sensing Agency, National Centre for Communicable Diseases, Forest, Agriculture, Animal Husbandry and Health Departments of State Governments, etc. The overall effort may be co-ordinated by a programme such as the Natural Resources Data Management System of the Department of Science and Technology¹⁶. Educational institutions should not only feed information into such a network, but should have access to information supplied by other agencies to facilitate students in taking projects using more synoptic information such as studies on geographical distribution patterns of their focal species.

Public awareness

Apart from becoming an integral part of the educational system, such an effort, based on the availability of good illustrated accounts of some 2500–5000 significant species of living organisms of the country could trigger off popular interest in the broader spectrum of the country's living diversity. At the time of independence there were

hardly any amateur bird watchers of Indian origin in our country; today there are hundreds of bird watchers' clubs scattered throughout the country. An important cause behind this transformation is the availability of good pictorial guides beginning with Sálím Ali's Book of Indian Birds first published in 1941. This has been followed by a series of other field guides often of excellent quality. To cite a few examples, these include Pascal and Ramesh¹⁷ on trees and lianas of Western Ghats, Tadulingam¹⁸ on Indian weeds, Mathur¹⁹ on coelomycetes, Daniel²⁰ on amphibians and reptiles, Whittaker²¹ on snakes, Gay, Kihimkar and Punetha²² on butterflies, Vijayalakshmi and Ahimaz²³ on spiders and Chhappargar²⁴ on seashore animals. In addition, there are the volumes of fauna of India and a number of excellent floras. But much of this latter material is too technical and of little practical use to non-specialists, even to Biology M Sc's. For instance, there is no ready aid for us to identify such striking groups of plants as lichens or of animals as dragonflies. None of this material also tells us how to go about assessing the populations of the species, or the many interesting questions that may be posed about their ecology, behaviour, distribution. The proposed material on the 2500–5000 species should fill these manifold gaps and make it possible for the citizens of India not only to become familiar with but ask intelligent questions about our living companions – not just birds, but butterflies and wasps, earthworms and orchids, starfishes and toads, lichens and lizards. If many of us can easily get to know them, their fate would become much more meaningful to us, contributing to a broader public concern for the health of India's environment. To serve this purpose, we must of course keep in mind what Sálím Ali was so notable for, namely, that 'besides providing factual scientific information to the reader it is just as important to make the account a pleasurable reading'²⁵.

Laying the foundation

Such an effort percolating the educational programmes at high school, college and university level, as well as promoting public awareness cannot of course substitute for the need to develop higher level skills and facilities to investigate the total range of country's biodiversity, at least two orders of magnitude higher than the proposed set of 2500–5000 species^{4,10,26,27}. But this effort would surely help attract more talented scholars and generate further public support for that effort. It would thus be an important foundation for this more advanced effort. At the same time it could in itself help create a very efficient system of monitoring the status of country's biodiversity, as well as programmes to control pests and vectors.

Organizing the effort

The Indian Academy of Sciences proposes to co-ordinate such a countrywide effort as a part of its initiatives to enhance the quality of science education, as well as publish scientific literature. It would work with a range of actors in this effort. These would include:

a) Systematic biologists with Botanical and Zoological Surveys of India, research institutions such as National Institute of Oceanography and with the Universities and Colleges.

b) Interested teachers at the High School, College and Post-graduate levels.

c) Institutions active in conservation and nature education such as Bombay Natural History Society, World Wide Fund for Nature – India, Centre for Environmental Education and C.P. Ramaswamy Foundation.

d) People's Science Movement groups such as Kerala Sastra Sahitya Parishat.

e) Knowledgeable practical ecologists, such as the tribal field guide Natarajan of the Annamalai Wildlife Sanctuary who brought the presence of Peninsular Bay Owl to the notice of scientists.

Interested people drawn from such groups should then be organized to decide upon and work towards the following objectives:

a) Deciding on the criteria to select the set of 2500–5000 species – or any other number agreed upon. These criteria should reflect adequate coverage in terms of: (i) taxonomic groups, (ii) geographical distribution, (iii) habitat preference, (iv) human significance in relation to economic, aesthetic, conservation or other considerations.

(b) Arriving at the actual list of species by applying these criteria.

(c) Deciding on what sort of descriptive/illustrative material should go into the account of each species. This could relate to morphology, habitat preference, seasonal changes, notable behaviour patterns, geographical distribution, relationship to humans, ways of discriminating from other related species, names in Indian languages, techniques for assessing population status, suggestions for student projects (see Appendix for a possible example).

(d) Commissioning the actual writing up of the accounts with the help of a panel of experts, which may include practical ecologists like Natarajan mentioned above.

(e) Peer review, revisions and editing of the prepared accounts.

(f) Translation of the accounts in Indian languages.

(g) Deciding on the form/s in which the material should be published and distributed. For instance, the material could be in several volumes on the model of

Ali and Ripley's *Handbook of Birds of India and Pakistan*. It could be supplied as loose-leaf material in ring files so that a high school, for instance in Shillong may only order the accounts of species present in Meghalaya prescribed for study by the State Department of Education. It could also be published electronically, as CD ROMS or on the internet.

(h) Actual production and distribution, hopefully so that at least some of the species accounts reach every high school, college or university, as well as homes of interested citizens throughout the country.

(i) Raising the funds for such a programme. This would especially involve honorarium for writing the accounts, preparation of illustrations and finally publication. At Rs 200 per species the writing and illustration would come to Rs 5 to 10 lakhs. If each account averages 3 pages, this would entail publishing 7500 to 15,000 pages. At Rs 300 per page this amounts to Rs 22.5 to 45 lakh. Another Rs 5 lakh may be required for co-ordination of the whole project, resulting in a budget of Rs 32.5 to 57.5 lakhs. Translation and publication in Indian languages would entail further expenses. It may be noted that these estimates are a small fraction of the three billion dollar budget projected for the grandiose systematics 2000 project, currently proposed as the basis of the effort at taxonomic capacity building for worldwide implementation of the Convention on Biological Diversity²⁸.

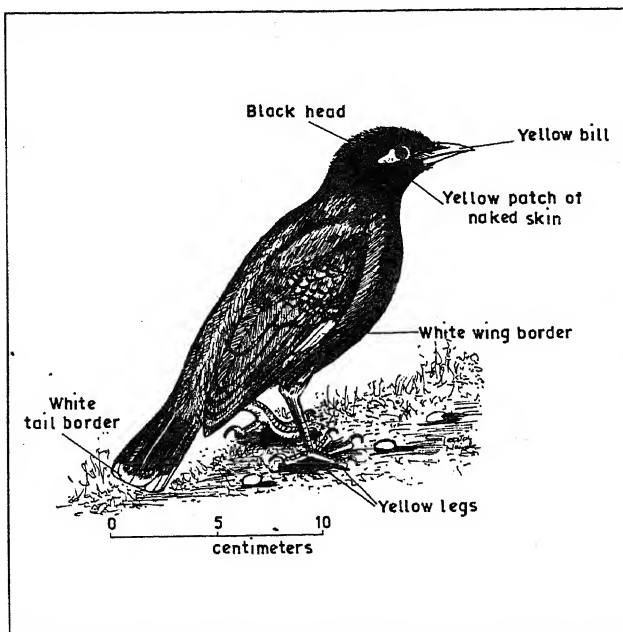
The Indian Academy of Sciences will be launching this programme in November 1996 to commemorate the birth centenary of Sâlim Ali, one of the Academy's most distinguished fellows. With this objective, the Academy is joining hands with the Indian Institute of Science and the Jawaharlal Nehru Center for Advanced Scientific Research to organize a discussion meeting that would bring together a group of over 100 people representing the many different sets of actors mentioned above. This meeting would debate on the specific content and time schedule for the project. But apart from this meeting suggestions, including offers of help and participation from all interested citizens of India; biologists, educationists, nature lovers would be most welcome. These should be addressed to the convener of the project, Madhav Gadgil at the Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560 012; Fax: 80-3341683; e-mail: madhav@ces.iisc.ernet.in.

APPENDIX 1

Indian Myna

Acridotheres tristis (Class Aves, Order Passeriformes, Family Sturnidae).

Size: 23 cm in length, about 110 gm in weight, a little smaller than a dove, bigger than a bulbul.



Field character: With its plump body, short tail and straight, sharp bill, the Indian Myna is a characteristic feature of Indian life. It is dark brown in colour with a glossy black head and bright yellow legs, bill and a naked patch below and behind eye. When in flight a white bar opens out on the wing; its tail is also bordered with white. Males and females are indistinguishable, the young are a little duller in colour with the heads ashy brown rather than black.

Related species. Indian Myna most resembles the Jungle Myna (*Acridotheres fuscus*) which lacks the yellow patch of skin behind the eye, is greyer in colour and has a little tuft of black feathers at the base of its bill on the forehead. The Bank Myna (*Acridotheres ginginianus*) is slightly smaller and pale bluish grey in colour, with a brick red patch of naked skin below and behind the eye.

Habits. Indian Myna follows people everywhere in the country, quick to colonize even far out in the desert. It is to be seen in town and villages, fields and gardens, sometimes walking after cattle, other times hunting insects on its own. It has a direct, business like flight in the air and a parade step on the ground. Mynas go in pairs or small parties, chattering a great deal. They sleep in large aggregations at communal roosts in large leafy trees, coconut groves, sugarcane fields, or in warehouses or railway stations. Such communal roosts are often mixed with those of crows, sparrows, parakeets or rosy pastors.

Nesting. The breeding season is primarily between April and July, but may commence as early as mid-January in Kerala and extend to September in parts of the country. Mynas nest in holes in trees or in walls and roofs of buildings. Usually there is considerable compe-

tition for nesting sites with violent fights between members of prospective pairs. Each partner grapples with its opposite number in a noisy rough and tumble, often dropping to ground. Mynas generally raise two successive broods over the breeding season laying clutches of 4 or 5 blue eggs.

Food. Indian Mynas have a broad range of diet, chiefly fruit, grain, insect and grubs but also small animals like baby mice, lizards and crabs and kitchen scraps from garbage dumps. They are also fond of nectar from bird flowers like silk cotton.

Distribution. Indian Mynas are resident, staying in a given locality year after year, probably coming to the same communal roost evening after evening. They occur throughout the subcontinent including Pakistan, Bangladesh, Nepal, Bhutan and Sri Lanka, going up to 3000 m in Himalayas. They have also been introduced to Andaman, Nicobars and Lakshadweep, as well as other parts of the world such as New Zealand.

Human significance. Indian Mynas are a companion of man all over the country amusing people with their chirpy chatter. To an extent they damage crops and orchards, but also help by destroying insects.

Population assessment. Indian Mynas are quite conspicuous and may be easily recorded on bird counts along straight transects. Their large noisy communal roosts with several hundred to thousands of birds may also be located and mapped, and birds counted fairly accurately as they gather at the roosts just before the sunset in the evenings. It is also possible to locate their nests and estimate their breeding populations in a given area.

Suggestions for student projects

- (1) Mapping of communal roosts of mynas, crows, parakeets and populations censuses; (2) Role of mynas in pollination of trees like silk cotton and coral trees; (3) Role of mynas as pests of crops like jowar; (4) Biological clock of Mynas in terms of time at which they return to the roosts in the evening; (5) Nesting success of Mynas; (6) Variety of calls used by Mynas in different situations.

Local names: Desi myna (Hindi); Hor (Kashmiri); Salik, Bhat salik (Bengali); Salik sorai, Salika, Ghor salika (Assamese); Dao myna (Cachari); Bemni, Saloo (Chota Nagpur); Gulgul (Madhya Pradesh); Shale, Salonki (Marathi); Kabar (Gujarati); Gorwantera (Kannada); Nahanavai (Tamil); Goranka (Telugu); Kavalamkili, Matatta (Malayalam).

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Common mechanism of secretory diarrhoea

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The studies of intestinal electrolyte processes have eluded the attempts to identify the common mechanism involved in secretory type of diarrhoea. The pathogenesis of secretory diarrhoea involves the coupled interactions of Ca^{2+} , PI, PKC, PGs, intramural nerves and cholinergic muscarinic receptor activation. These interactions may result in increase in intracellular Ca^{2+} levels. The increased Ca^{2+} may thus serve an important intracellular mediator involved in the stimulation of intestinal fluid secretion.

THE worldwide impact of acute infectious diarrhoeal diseases is immense, with an estimated 3–5 billion cases per year, resulting in 3–5 million deaths each year¹. A survey reported more than 3 million deaths each year due to diarrhoea². Eighty per cent of these deaths occur in children below two years of age³. It is estimated that the children less than two years of age in developing countries suffer 6–10 episodes of diarrhoea per year, thus approximately 17% of their life in this age group is spent with diarrhoea⁴. A close scrutiny of facts and figures available from National and International sources indicates that first two years of life of a child born in India continues to be crucial from health point of view. It has been estimated that approximately 3 million children die before completion of one year and one million die before attaining childhood every year and these deaths in one way or other are related to diarrhoeal diseases and their effects⁵.

Infectious diarrhoea is generally secretory in nature and can result from bacterial, viral and parasitic infections. The bacteria commonly implicated in the etiology of diarrhoea include conventional enteropathogens like *Shigella*, *Salmonella*, enteropathogenic *E. coli* (EPEC), *Vibrio cholerae* and new enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), *Yersinia*, *Aeromonas*, campylobacter and recently isolated *Vibrio* 0139 synonym Bengal^{6–9}.

The understanding of underlying pathophysiological mechanisms of acute secretory diarrhoea is growing steadily, now thanks to the intense research interest in the physiology of intestinal electrolyte transport. Considerable insights have been obtained with respect to normal cellular mechanisms of ion transport, though the studies of intestinal electrolyte transport are complicated by several factors (Table 1).

Structural basis for intestinal electrolyte transport

The epithelium of small intestine is located at the interfacial surface, the intestinal lumen is in continuity with external environment. The material absorbed from lumen must first traverse this epithelium to gain access to mucosal blood and lymph vessels.

Structural organization of mucosa of small intestine

The luminal surface of small intestine is so organized that surface area is greatly amplified. Numerous microscopic mucosal villi increase the absorptive surface some seven to 14-fold¹⁰. Depending on the mammalian species and portion of small intestine, villus height and shape vary. Diseases that affect the mucosal function often perturb normal villus structure, thus affecting absorptive surface of small intestine¹¹.

The intestinal mucosa is divided into three distinct layers (Figure 1). The deepest is the muscularis mucosa that separates mucosa from submucosa. Its contractile properties help in the movement of villi and emptying of crypt luminal contents¹². The lamina propria is the middle mucosal layer, which is bounded above by epithelium and below by muscularis mucosa. The lamina contains eosinophils, mast cells, fibroblasts, unmyelinated nerve fibres, blood and lymph vessels. The lamina propria provides support to intestinal epithelial cells and also contains blood vessels that nourish the epithelium. The third layer of intestinal mucosa constitutes a continuous sheet of epithelial cells which line the villi and crypts. The crypt epithelium is composed of undifferentiated cells, mucus secreting goblet cells, endocrine

Table 1. Regulation of cellular mechanisms of ion transport

1. Presence and simultaneous function of both absorptive and secretory processes.
2. Transmucosal movement of ions through both cellular and transcellular pathways.
3. Presence of multiple cell types.
4. Structural heterogeneity of epithelial cells.

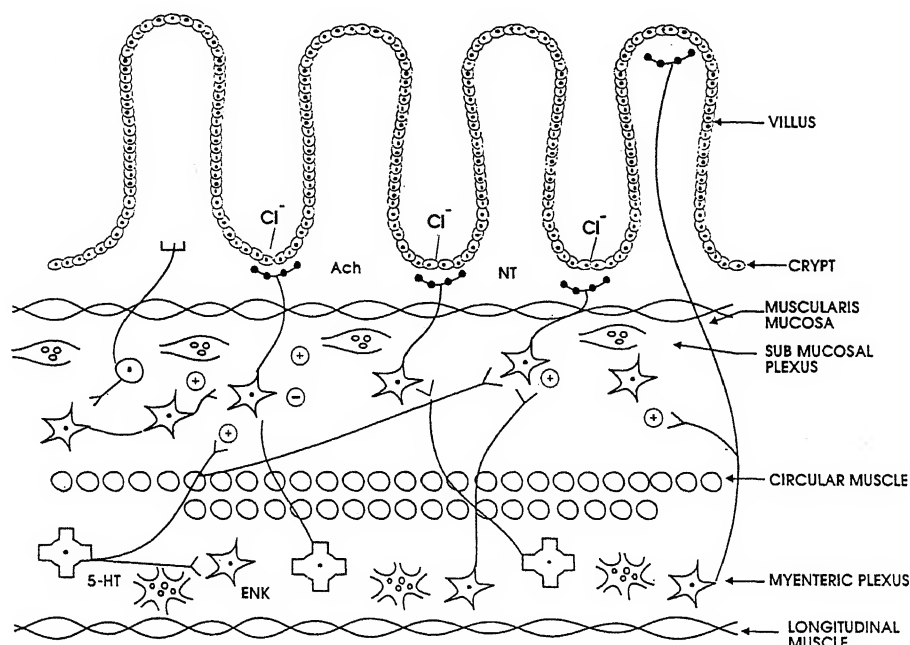


Figure 1. Functional morphology of mucosa of small intestine.

epithelial cells, tuft cells and Paneth cells with secretory granules. The epithelium lining the villi contains absorptive cells, termed as enterocytes, mucus secreting goblet cells, few endocrine epithelial cells, rare caveolated cells, cup cells. Additionally, a specialized epithelial cell, the M cell overlies the apex of Peyer's patches.

The functions of crypt epithelium include epithelial cell renewal, secretion into crypt lumen, electrolyte and water secretion and endocrine secretion both into the lamina propria and into the lumen. The major known function of villus epithelium is absorption of nutrients¹².

Cellular mechanisms of ion transport

Na^+ and Cl^- together are by far the major ions of fluid transported during absorption or secretion by small intestine. Na^+ , in particular, plays a central role in the energetics of the intestinal absorption via the transcellular route. The fluid and electrolyte transported depend upon the net effect of absorptive and secretory processes.

Na^+ absorption mainly occurs by three mechanisms (Table 2). The driving force for Na^+ entry is the hydrolysis of ATP catalysed by Na^+ , K^+ -ATPase located at basolateral membrane of enterocytes¹³. This pump exchanges Na^+ out of K^+ in, thus establishing a steep electrochemical gradient for Na^+ entry from lumen (Figure 2). The small intestine of most mammals also possesses a secretory mechanism¹⁴, which normally functions at a low basal level (Table 2). Previous findings support a distribution of secretory function to the crypt cells^{15,16}. Recently, it has been demonstrated that crypts also

Table 2. Ion transport processes

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|--|
| I. Absorptive processes |
| 1. Electrogenic sodium absorption |
| 2. Non-electrolyte stimulated Na^+ absorption |
| 3. Electroneutral sodium chloride absorption |
| II. Secretory processes |
| 1. Sodium secretion |
| 2. Chloride secretion |

absorb fluid and Cl^- secretion occurs in presence of intracellular or extracellular messengers¹⁷.

The mechanisms of electrolyte transport differ in various segments of mammalian intestine. In jejunum, Na^+ entry occurs by (i) Na^+/H^+ exchange, (ii) Na^+ substrate cotransport, (iii) $\text{Na}^+-\text{PO}_4^-$ and $\text{Na}^+-\text{SO}_4^{2-}$ co-transport but active Cl^- absorption does not occur, there is no evidence of direct linking of Na^+ and Cl^- transport, and no $\text{Cl}^-/\text{HCO}_3^-$ or Cl^-/OH^- exchanger¹⁸. In mammalian ileum, Na^+ and Cl^- transport are linked and Na-substrate mechanism is less prominent¹⁹. The mammalian distal colon demonstrates amiloride sensitive Na^+ absorption pathway whereas proximal colon possess a coupled NaCl transport mechanism²⁰.

Pathophysiological mechanisms in diarrhoeal diseases

Cyclic nucleotides

Cyclic nucleotides stimulate intestinal secretion by three potential mechanisms: (i) altered activity of ion trans-

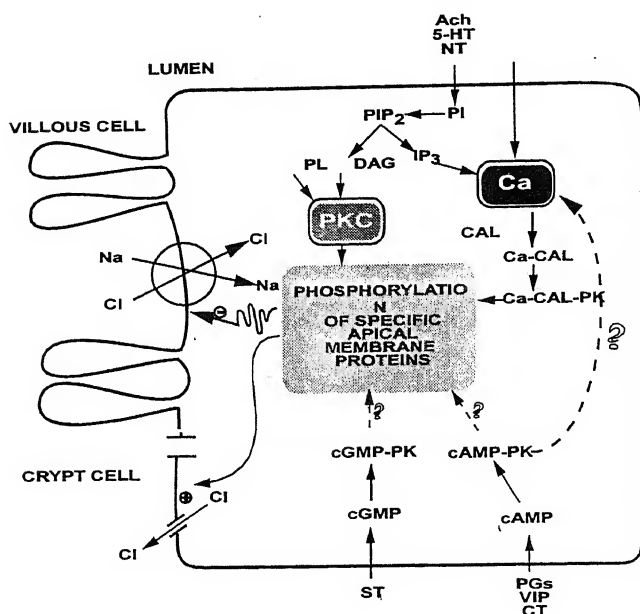


Figure 2. Regulation of intestinal secretion by extracellular mediators and intracellular messengers.

porters, (ii) modulation of tight junctions, and (iii) increase in intracellular calcium levels.

The elevation of cAMP or cGMP levels in intestinal epithelial cells stimulates active chloride secretion by inhibiting electroneutral NaCl absorption in intestinal epithelium²¹. Cyclic AMP acts via stimulation of A kinase with direct phosphorylation and activation of the major chloride channel identified in intestinal epithelial cells, the cystic fibrosis transmembrane conductance regulator (CFTR)²². Second, cyclic AMP regulates intestinal epithelial cell secretion via effects on cytoskeletal proteins. The chloride secretory response to cAMP is dependent on microtubules²³. Further, the apical membrane recruitment of CFTR in T84 cells in response to cyclic AMP is dependent on microtubules but not calcium or F (filamentous) actin²⁴. The mechanism by which activation of cAMP reorganizes F actin is not known. Besides its effect on ion transporters, the study on CT-treated animal tissues, *Campylobacter jejuni* and *Salmonella typhimurium* heat labile toxin suggest that cyclic AMP potentially contributes to intestinal secretion²⁵⁻²⁷.

Lastly, cyclic AMP-dependent agonists stimulate increases in intracellular calcium (Ca^{2+})_i in T84 intestinal epithelial cells. The cAMP-mediated increase in (Ca^{2+})_i occurs via a pathway (Figure 2) distinct from that mediated by inositol triphosphate (IP_3) or protein kinase C (PKC)^{28,29}.

Intracellular calcium

There are several potential mechanisms by which ele-

vation in (Ca^{2+})_i levels stimulates a net intestinal secretion. The elevation of (Ca^{2+})_i levels alters the regulation of several ion-transporting proteins. The increased (Ca^{2+})_i may activate calmodulin-dependent protein kinases (Ca-CaM) or calcium-dependent protein kinase (PKC)³⁰. These activated kinases may phosphorylate membrane proteins that affect the activity of apical membrane Na^+/H^+ exchanger (Figure 2) resulting the decreased Na^+ absorption³¹. Secondly, Ca-CaM dependent protein kinases and PKC may activate CFTR³². Undoubtedly, elevated calcium is enough signal to stimulate chloride secretion in intestinal epithelial cells in the absence of additional second messengers. The previous studies based on evaluating the role of calcium in the presence of its agonists and antagonists suggest its involvement in pathogenesis of many diarrhoeagenic organisms^{26,33-36}. Additionally, calcium has been known to regulate tight junctions, suggesting that changes in (Ca^{2+})_i levels modulate the intestinal permeability and contribute to intestinal secretion³⁷.

Protein kinase C

The PKC family consists of multiple isoenzymes that differ from each other in one way or other³⁸. Specific isoforms of PKC are present in various intestinal cell types. The metabolism of phosphatidyl inositol by phospholipase C (PLC) results in generation of IP_3 and diacylglycerol (DAG). IP_3 releases calcium from intracellular calcium stores whereas DAG activates PKC. The role of PKC activation in stimulating intestinal secretion has been examined by use of activators and inhibitors of PKC with help of Ussing chambers^{26,35,39}. Besides causing intestinal secretion, PKC activation also leads to disassembly of intestinal epithelial cell cytoskeleton and modulation of tight junction⁴⁰. Thus it suggests that PKC activation may contribute to intestinal secretion through direct effects on ion transporters as well as through regulation of paracellular transport pathway.

Phospholipases

The activation of phospholipases leads to changes in intracellular mediators which regulate transport of fluid and electrolytes. The most studied examples are (i) release of arachidonic acid metabolites by the activation of PLA_2 and (ii) release of intracellular calcium through activation of PLC. Activation of PLA_2 leads to the release of AA metabolites, viz. prostaglandins (cyclooxygenase pathway products) and leukotrienes (lipoxygenase pathway products). Certain prostaglandins and leukotrienes may serve to amplify the secretory response for example, prostaglandin I_2 (PGI_2) can activate enteric nervous system and leukotriene B4 stimulates the

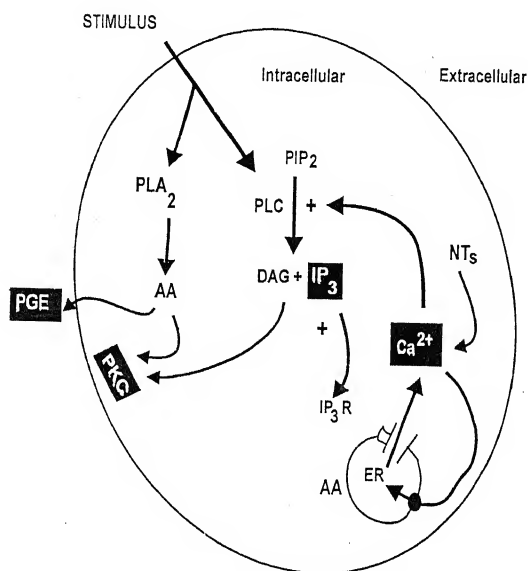


Figure 3. Proposed pathophysiological mechanism(s) involved in secretory diarrhoea.

recruitment of PMNs, leading to an inflammatory response in intestine. Activation of PKC regulates ion transport through the release of calcium and PKC activators as summarized above⁴¹. Recently, additional signalling pathways that involve the activation of PLD and phosphatidyl specific PLC have been identified^{38,42}. These pathways lead to the release of lipid metabolites (e.g. diacyl glycerol and phosphatidic acid) that regulate specific cell functions.

Enteric nervous system

Considerable evidence exists regarding the involvement of one or more components of enteric nervous system in secretory responses to bacterial toxins⁴³. Neural pathways appear to involve villus to crypt neural reflexes. The neuropeptides (Figure 1) such as those secreting substance P, 5-HT, vasoactive intestinal peptide (VIP)^{44,45} and acetylcholine have been implicated in the secretory responses to *C. difficile* toxin A, CT, *E. coli* heat stable enterotoxins (STa and STb) and Shigella toxin^{36,46-48}. Several bacterial pathogens and/or their toxins have been shown to alter intestinal myoelectric patterns. These include CT, *E. coli* STa, wild type and recombinant *V. cholerae* strains, Shigella toxin and various classes of *E. coli* [e.g. enteropathogenic *E. coli* (EPEC), ETEC and enteroinvasive *E. coli* (EIEC)]^{36,49-51}.

Summary

Diarrhoea is one of the major causes of mortality during infancy and early childhood in developing countries.

The underlying pathophysiological mechanisms in secretory diarrhoea involves the complex interaction of several biochemical pathways. Calcium may be located at the cross over point of various pathways involving PKC, prostaglandins, intramural nerves and cyclic nucleotides. Increased calcium (either through extracellular calcium or release of calcium from intracellular stores) may activate PKC by its translocation from cytosolic to membrane, thereby phosphorylate membrane proteins. The sequence of interaction leading to an increase in intracellular Ca^{2+} appears central to the control of diarrhoea modulating ion transport through activation of protein kinase C (Figure 3).

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Molecular biology and biotechnology of higher plant nitrate reductases

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Nitrate reductase (NR) catalysing the reduction of nitrate to nitrite is the first and rate limiting enzyme in the assimilation of nitrate by the plants. At least two of its isoforms; a NADH specific and a NAD(P)H bispecific have been characterized. In this article, the physico-chemical properties of NADH specific isoform, which is the principal isoform of nitrate assimilation, have been described. The properties of cloned genes and the production of transgenic plants with altered NR activity, and mechanism of induction of NR by nitrate and cytokinins, repression by glutamine and post-translational modification of NR protein through reversible phosphorylation by light-dark transitions have also been accounted for. The characterization of NAD(P)H : NR gene and some regulatory aspects of this isoform have also been described. The article demonstrates that it is possible to produce mutants and transgenics with altered structure and function of NR, with an ultimate aim to affect qualitative and quantitative improvement of crop plants.

NITRATE reductase (NR) catalysing the reduction of nitrate to nitrite is the first enzyme in the assimilation of nitrate

by plants. The activity is considered to be the rate-limiting step in nitrate assimilation, which is often positively correlated with the total protein and nitrogen contents and sometimes also with the overall productivity of the plants¹. Three isoforms of this enzyme have been described from soybean², viz. (i) A nitrate inducible NADH : NR (E.C.1.6.6.1) with a pH optimum of 7.5, (ii) A constitutive or inducible bispecific NAD(P)H : NR (E.C.1.6.6.2) with a optimum pH of 6.5, and (iii) A constitutive NADH : NR (E.C. number not yet assigned), with a pH optimum of 6.5. The inducible NADH : NR and the bispecific NAD(P)H : NR are usually found in close association with various plants and perhaps both are involved in nitrate assimilation. The NAD(P)H : NR however, is considered to be associated with some other functions also, such as with the transport of nitrate across the membranes and with the dissimilatory release of oxygen in anaerobic environment³. The evidences for these alternate functions are yet to be known, although the observation that NAD(P)H : NR isoform was localized principally on the plasma membrane⁴ is a strong indicator of its role in nitrate acquisition and transport. NR has been used as a biotechnological tool/product also. Mellor

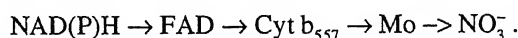
*et al.*⁵ have evolved a bioreactor that employs immobilized NR from maize along with some bacterial enzymes to reduce nitrate to nitrogen gas. The bioreactor may be used for reducing nitrate contamination of groundwater which is otherwise rendered unfit for human consumption because of toxic nitrate contamination. Similarly, the accumulation of toxic levels of nitrate in crop plants can also be restricted by *in vivo* regulation of NR through genetic manipulations. For example, Quillere *et al.*⁶ have demonstrated that genetically transformed *Nicotiana plumbaginifolia* plants with a 25 to 150% higher NR activity have a 32 to 47% lower nitrate contents as compared to wild type controls. Thus, the interest in the study of NR has been generated with different objectives.

Physico-chemical nature of the enzyme

Although the NR from higher plants was characterized for the first time in the early fifties⁷ its purification could be achieved only in the seventies^{8,9}, primarily because the enzyme is very unstable and it requires special care for extraction, isolation and purification. The *in vivo* experiment showed that the protein is short lived with a half life of only 3 to 5 hours¹⁰. The enzyme has been purified from a variety of species including barley, maize, soybean, spinach, squash, tobacco, etc. by affinity chromatography and electrophoresis. Monoclonal antibodies of the enzyme have been raised^{11,12}. Nitrate reductase (E.C. 1.6.6.1) from higher plants is a homodimer with each sub unit of 100 to 120 kD molecular mass of 881 (bean) to 926 (spinach) amino acids¹³. In each sub unit, acidic amino acids are more abundant than the basic ones; the isoelectric point of a squash NR has been determined¹⁴ to be 5.7. There are 9 to 19 cysteine residues in each sub unit and the two sub units are linked through at least three disulphide bonds of cysteine residues¹⁵. Each sub unit contains at least three prosthetic groups, FAD, heme (cytochrome b_{557}) and Mo-pterin (Mo-CO). The NADH/NADPH binding region and the FAD domain are at C-terminal while Mo-CO is at N-terminal of the polypeptide. The cytochrome heme domain occupies a central part in the polypeptide. The Mo-pterin domain is a complex of Mo atom linked to a pterin (a heterocyclic compound) molecule via a thiol group (Figure 1). The three domains of the polypeptide are apparently linked through two hinges; hinge I (HI) between Mo-Co and cytochrome and hinge II (HII) between the cytochrome and the FAD. As described later HI plays an important role in the enzyme modulation by phosphorylation-dephosphorylation¹⁶. The three regions of the polypeptide can be separated by treatment of the enzyme with appropriate proteases. Three separated domains show partial activities

of the enzyme. The Mo-Co domain is believed to be involved in the dimerization of sub units.

The reductant used in the reduction of nitrate to nitrite is NADH. The bispecific NAD(P)H : NR however, uses either NADH or NADPH; the preference being for the latter. The initial acceptor of the electron from NADH/NADPH is FAD of the enzyme. Then the electron flows through cytochrome b_{557} site to Mo-pterin site in the enzyme and ultimately to the nitrate, which is reduced to nitrite;



Active site amino acid mapping of the enzyme has been done by using appropriate enzyme inhibitors. Histidine¹⁷ and arginine & lysine¹⁸ are involved in NADH binding with the enzyme. A difference in the amino acid sequence between bispecific NAD(P)H : NR of birch (*Betula pendula*) and that of monospecific NADH : NR of many species have been found in the FAD domain of the enzyme¹⁹. Arginine residues are involved in the catalytic function of FAD and Mo-CO domain²⁰. Specific inhibitors binding these amino acids block the flow of electrons from NADH to nitrate and hence the catalytic activity of NR. Cysteine also appears to be actively participating in the electron transfer. Dwivedi *et al.*²¹ replaced each of the five cysteine residues of recombinant cytochrome reductase domain from maize leaf NR with other amino acid analogues using site-directed mutagenesis. The enzyme had intact NADH-binding sites but had reduced ferricyanide reductase activity. The authors have concluded that cysteine is essential for a highly efficient catalytic transfer of electrons from pyridine nucleotides to flavins.

Gene cloning and transgenics

Genetic experiments including the study of NR deficient mutants have helped in the identification of genes coding for the synthesis of NR protein. In barley, two structural loci *Nar 1* and *Nar 7* have been identified, which

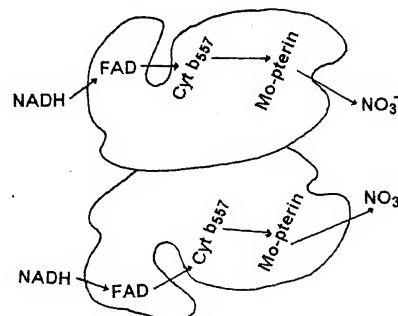


Figure 1. Diagrammatic representation of the structure of nitrate reductase showing path of electron transfer.

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apparently code for inducible NADH:NR and NAD(P)H:NR respectively²². Two genes *NR 1* and *NR 2* have been identified in *Arabidopsis thaliana*²³ and in tobacco²⁴. Zhou *et al.*²⁵ have described the presence of only one gene for NADH:NR in cultivated barley, while two genes in diploid wheat *Triticum monococcum* and in hexaploid wheat *Triticum aestivum*. They have suggested that during evolutionary events NADH:NR gene duplicated before the divergence of wheat and barley and that a deletion resulted in the loss of one NADH:NR gene from cultivated barley.

Both the NADH and NAD(P)H specific NR genes have been now cloned from a variety of species from either genomic library or from c-DNA (Table 1). The complete sequence of the nucleotides is known from *Arabidopsis*, birch (NAD(P)H:NR), barley, bean, rice, tobacco and tomato¹³. The NADH:NR gene is about 3000 to 5000 bp long and contains about one to three introns. The genome of barley NADH:NR is however, longer, about 7300 nucleotides and the NAD(P)H:NR from the same species is 3538 bp with two introns. A

transposable element of about 565 bp in the first exon of NR genome of a NR-deficient mutant (obtained by γ -irradiation) of tobacco has been detected²⁶. It occurs as a single copy in the genome and is apparently lacking in *Lycopersicon* and *Petunia*.

Transgenic tobacco with altered NR activity has been developed²⁷⁻³¹. Constructs carrying the entire or part of the tobacco c-DNA cloned between the promoter and terminator sequence of the 35 S RNA of cauliflower mosaic virus were introduced in tobacco²⁷. A few NR-deficient transgenics were obtained and a few with increased NR m-RNA and NR protein were also obtained. In another attempt, NR deficient tobacco plants were transformed to high NR activity types through the transfer of NR c-DNA with 35 S constitutive promoter from cauliflower mosaic virus²⁸. The transgenics accumulated higher amounts of glutamine and reduced levels of nitrate in their leaves. Transgenic tobacco have helped in visualizing the physiological significance of the inducibility of NR protein by nitrate. The study has shown that the constitutive expression of NR does not influence

Table 1. Some characteristics of nitrate reductase genes cloned from higher plants

Plant species and the organ	Clone	Some characteristics	Reference
A. NADH:NR gene			
<i>Arabidopsis thaliana</i> leaves	Genomic	3829 bp, two introns	83
<i>Cucurbita maxima</i> (squash) cotyledons	c-DNA	2754 bp plus a untranslatable region of 135 nucleotides at 3' end and poly A tail of 16 nucleotides, coding for 918 amino acids	84
<i>Glycine max</i> (soybean) leaves	c-DNA	<i>NR 1</i> : 2661 bp, coding for 886 amino acids <i>NR 2</i> : 2673 bp, coding for 891 amino acids	46
<i>Hordeum vulgare</i> (barley) leaves	Genomic	7300 bp, one large intron of 2700 bp, coding for 915 amino acids	41
<i>Lycopersicon esculentum</i> (tomato) leaves	Genomic	5300 bp, three introns, coding for 911 amino acids	85
<i>Nicotiana tabacum</i> (tobacco) leaves	Genomic	6000 bp, three introns coding for 904 amino acids	86
<i>Oryza sativa</i> (rice) leaves	Genomic	5400 bp, three introns coding for 916 amino acids	87
<i>Phaseolus vulgaris</i> (bean) - roots	Genomic	4600 bp, three introns, coding for 881 amino acids	88
- leaves	Genomic	Four introns, coding for 890 amino acids	89
<i>Spinacea oleracea</i> (spinach) leaves	c-DNA	3284 bp, two introns, coding for 926 amino acids	90
B. NAD(PH):NR gene			
<i>Betula pendula</i> (birch) leaves	c-DNA	3031 bp, coding for 898 amino acids	91
<i>Hordeum vulgare</i> (barley) leaves	Genomic	3538 bp, coding for 891 amino acids	42

the foliar protein and chlorophyll contents, the assimilatory products of nitrate, under any circumstances²⁹. Further, under the conditions of nitrogen deficiency, the NR protein is actively degraded.

Transgenics have also been created with a view to understanding some of the regulatory aspects of NR. Nussaume *et al.*³¹ have transformed a NR deficient tobacco by introducing a full length *Nicotiana* c-DNA with an internal deletion of 168 bp in the 5' end fused to cauliflower mosaic virus 35 S promoter and appropriate termination signals. In transgenic plants expressing this construct, NR activity was restored and normal growth resulted. However, *in vitro* NR activity from these transformants was not modulated by ATP and light. A heterogenous expression system has been developed to examine the regulatory mechanism of NR through phosphorylation-dephosphorylation³². Wild type and mutant forms of NR c-DNA from *Arabidopsis thaliana* have been inserted and expressed in the yeast *Pichia pastoris*. This allowed to produce mutant forms of NR for various regulatory studies. The technology may also be used in the production of plant NR in bulk for X-ray crystallography and other biophysical investigations.

Transcriptional control of NR gene expression

Nitrate reductase is subjected to a multivalent control at gene expression level³³. Some of the regulators of the enzyme are structurally or metabolically related to nitrate, the substrate of the enzyme, or to one of the assimilatory products of nitrate; while others are general regulators of NR and of other enzymes.

Induction by nitrate

Induction of NADH:NR gene has been demonstrated by nitrate, cytokinin and light. Nitrate is the substrate of the enzyme and also a specific inducer of NR activity, a property which has been demonstrated in almost all the investigations¹. Different levels of nitrate are required for optimum induction of NR in different species, which is perhaps linked to the differential rates of uptake and/or mobilization of nitrate to the genomic level¹. When the entire plant or its excised organs are incubated in a nitrate-containing medium, the NR activity in the plant or organ starts increasing after a lag of about 15–45 min and goes on increasing almost linearly until 180–400 min. Thereafter, a steady state level is maintained. However, if nitrate is withdrawn from the medium, the steady state level declines. Further, the linear increase in enzyme activity is abolished in the presence of transcriptional and translational inhibitors. Chemical isolation of RNA, Northern blotting and c-DNA isolation and hybridization experiments performed in a variety of

plant systems have demonstrated that nitrate supply in fact increased the synthesis of m-RNA. For example, in *Cucurbita maxima* (squash) an increased apoprotein synthesis in response to nitrate was linked with the increase in m-RNA³⁴. This increased transcription required intact plastids in the cotyledons³⁵. In barley, when nitrate-starved seedlings were transferred to a nitrate-containing medium, NR m-RNA increased rapidly from essentially zero to high levels within a few hours in both roots and shoots³⁶. In nitrate cultured tobacco and tomato seedlings also, further supply of nitrate increased NR m-RNA, although at relatively slow rate³⁷. In maize roots, the increase in NR activity in response to nitrate is correlated with the increase in NR m-RNA^{38,39}. Transcriptional assays with isolated soybean nuclei also indicate that induction of NR m-RNA is due to *de novo* synthesis of the transcript and not due to reduced RNA degradation.

In a recent study, a nitrate independent transcription of two NR isogenes in androgenic haploid embryos of *Brassica napus* (rape seed) has been demonstrated⁴⁰. However, in the leaf, NR gene expression responds to nitrate supply as in most other systems. This demonstrates the uniqueness of the regulation of NR gene expression in haploid embryogenesis.

The effect of nitrate supply on NAD(P)H:NR has also been examined. In barley, the genes for both NADH:NR and NAD(P)H:NR have been isolated and sequenced^{41,42}. There is a high degree of sequence similarity between the two genes. However, there is a significant divergence at 5' end of the gene and thus it has been possible to differentiate between the m-RNAs of two genes. The divergence also indicates that there was a possibility of differential regulation of the expression of two genes. In soybean, nitrate supply has no effect on NAD(P)H:NR activity although it induces NADH:NR^{2,43}. However, in barley, NADH: and NAD(P)H:NR m-RNAs are at detectable levels in both roots and leaves within 15 to 30 min after exposure of seedling roots to nitrate⁴⁴. Nitrate depletion triggers a rapid decrease in both m-RNAs and in the activities of both the isoforms. It indicates that in barley at least both the genes, *Nar 1* and *Nar 7* coding for NADH:NR and NAD(P)H:NR respectively are under the control of the same promoter/regulator. Vaucheret and Caboche³⁰ by using transgenic *Nicotiana plumbaginifolia* have demonstrated that the promoter for *Nia* gene is specific for the gene. In transgenic plants, the expression of a reporter gene neomycin phosphotransferase driven by a 1.4 kb *Nia* promoter had a negative correlation with the induction of *Nia* gene³⁰. Positive response of NAD(P)H:NR to nitrate supply has been demonstrated in maize also³⁸, although the species has a significant level of constitutive expression also (Shankar and Srivastava, unpublished).

The mechanism of signal transduction in nitrate induction of NADH or NAD(P)H : NR gene is not known. Presumably *cis* acting elements and *trans* acting proteins are involved in the induction. In transgenic tobacco plants containing *Arabidopsis* NR genes, the DNA sequence responsive to nitrate has been identified to be at 5' end, a 238 or a 188 bp sequence in NR 1 or NR 2 genes respectively⁴⁵. The promoter region for the two genes in tobacco, however seems to be larger²⁴.

Regulation by glutamine

Inhibition of NR activity by the addition of glutamine has been demonstrated in many species. That the inhibition is at transcription level has been demonstrated in soybean^{40,46}, tobacco^{47,48}, maize¹⁰ and in cultured spinach cells⁴⁹. The inhibition is partially overcome by the addition of glucose in tobacco leaves⁴⁸. Endogenous glutamine level also seems to be controlling NR gene expression. In tobacco, inhibition of glutamine synthetase enzyme leads to a sharp decline in glutamine level, which resulted in an increased NR m-RNA⁴⁷. In maize seedlings, the root enzyme is more sensitive to exogenously supplied glutamine than the shoot enzyme, which is apparently because of the more sensitive NAD(P)H:NR (primarily a root enzyme) than the NADH:NR³⁹. However NR m-RNA was almost equally inhibited by glutamine in both roots and shoots. Thus, there might be some additional aspect of down regulation of active NR protein formation by the glutamine in the roots.

Induction by cytokinins

Exogenous supply of cytokinins invariably increases NR activity, although the magnitude of increase depends upon the species and the concentration and nature of the cytokinin. Apparently, the endogenous cytokinin level is an important factor in determining the effects of exogenous cytokinins. As with nitrate, cytokinins also appear to be acting at transcription level in inducing NR activity^{50,51}. Adenine or adenosine have no effect on NR activity⁵¹. The induction by cytokinins is usually higher in the presence of nitrate than in its absence⁵², although the two inducers do not seem to be acting synergistically⁵¹. Samuelsson *et al.*⁵¹ have also demonstrated that nitrate supply elevated cytokinin zeatin riboside level in barley roots and shoots. This could determine the influence of externally supplied cytokinin on NR activity in the presence of nitrate. The exact mechanism of induction of transcription by cytokinins is not known. However, Suty *et al.*⁵³ have reported that cytokinin-mediated transcription in tobacco cell suspensions involved m-RNA polyadenylation.

The plant hormone abscissic acid represses NR gene expression in barley, which is partially recovered by equal concentration of benzyladenine⁵⁰. Thus, abscissic acid also might be acting at transcription level. Bueno *et al.*⁵⁴, however, have suggested that kinetin-mediated increase in NR activity in *Cicer areitenum* cotyledons is both through synthesis and activation of the enzyme.

Effect of light and sugars

Light is another factor regulating NR activity in upward direction. Enzyme extracted from the organs of the plants raised in light is several folds more active than those from dark grown. The positive response of light is apparent in non-photosynthetic organs such as roots, endosperm and scutella also⁵⁵. In many systems, the effect of light appears to be at transcription level, although post transcriptional modifications of NR protein by light is also known.

In a study with five-day-old maize seedlings, Lillo⁵⁶ observed a four-fold increase in NR m-RNA level within one hour exposure to light. This was reported to be due to increased transcriptional activity. In another study with maize, Huber *et al.*⁵⁷ also reported a significant increase in leaf NR m-RNA within two hours of illumination of previously darkened seedlings.

It has been often suggested that the light/dark effects on NR activity were responsible for observed diurnal variation in enzyme activity. However, in a few investigations, an increase in NR m-RNA and in NR activity has been observed during the night. This is apparently because sucrose and glucose replace light requirements in the induction of m-RNA as has been reported in *Arabidopsis*²³ and tobacco⁴⁸. In tobacco leaves, fructose also induces transcription of NR m-RNA while ribose or mannitol has no effect⁴⁸. This has led these investigators to believe that up-regulation of NR gene transcription by light is mediated via carbohydrate synthesis in green leaves.

There is also some indication of post transcriptional modification of NR m-RNA in plants. In *Arabidopsis*, the expression of *Nia 2* gene shows circadian oscillations⁵⁸. The m-RNA accumulation also shows rhythm even up to five days in continuous darkness after the plants have been grown in light/dark cycle.

Translational control

The modification of NR protein synthesis at translation level has not been demonstrated although it is often assumed that many factors controlling transcription have effect on translation also. There is some circumstantial evidence for translational control of NR synthesis by oxygen, which is known to inhibit NR activity in oat

leaves⁵⁹. In detached maize leaves, NR activity and NR protein appearance were partially inhibited at 100% oxygen, but the m-RNA levels as measured by leaf NADH:NR c-DNA hybridization tests were the same in air and 100% oxygen-treated leaves⁶⁰.

Post-translational modification of NR protein

Some of the regulators of NR activity have very fast effects, the activity responding the regulator as quickly as in a few minutes. These regulators apparently act through the modulation of NR protein. For example, in spinach leaves, the NR activity decreases rapidly during darkening of the leaves, reaching to about 15% of the control value in only about two minutes⁶¹.

The processing of the nascent NR protein into an active NR molecule itself is a step which is affected by some regulators. Molybdenum cofactor is inserted in the NR protein after it has been fully synthesized. In the absence of Mo, no active NR is formed. Tungsten, a metal classified with Cr, Mo and U in the periodic table competes with Mo, and produces a NR protein which is inactive in nitrate reduction⁶².

Reversible phosphorylation

The reversible phosphorylation of NR protein appears to be the most important mechanism of enzyme regulation in both prokaryotes and eukaryotes. It allows a fast modulation of enzymic protein with the change in its micro-environment. For NR, it has been now demonstrated that phosphorylation-dephosphorylation was involved in dark-light inactivation/activation of the enzyme. Upon transition of plant or plant organs from darkness to light, the NR protein is in dephosphorylated (active) form, whereas phosphorylation of specific serine residues is increased on transition to darkness^{63,64}. The phosphorylation is catalysed by specific protein kinases; two protein kinases, NR:PK I and NR:PK II have been isolated from spinach leaves⁶⁵. NR:PK I has a broader specificity and can phosphorylate sucrose phosphate synthase also. NR:PK II however, which has an apparent *Mr* of 160 kD, is specific for NR only. The NR specific PK (PK II) is a Ca^{2+} -dependent and uses ATP as phosphorylation substrate. *In vitro* experiments have revealed that γ -³²P of ATP is incorporated into NR protein from *Brassica campestris*⁶⁶. Analysis of phosphoamino acids in phosphorylated NR from *Arabidopsis* has revealed that serine at position 308 in Mo-Co domain is required for phosphorylation of NR and also apparently for normal activity of the enzyme⁶⁷. When serine is replaced by aspartic acid, both the processes, the normal activity and the phosphorylation of the NR protein are disrupted. However, in spinach, phosphorylation of Ser-

543 has been demonstrated by using recombinant DNA fragments²⁴. In another investigation with *Arabidopsis* NR expressed in *Pichia pastoris*, Ser-534 is shown to be phosphorylated³². In both these systems, Ser-543 or Ser-534 are located in hinge I region and occupy equivalent position in the polypeptide. Further, transgenic tobacco containing a 168 bp deletion at 5' end of the *Nia* are insensitive to modulation by ATP or light³¹. However, it is not clear whether this deletion results in the deletion of the critical serine residue or in some other kind of amino acid changes. The phosphorylation of NR protein in dark is apparently not itself enough for complete enzyme inactivation. The inhibition in fact is affected by another protein known as inhibitor protein (IP; *Mr* 110 kD) which in the presence of Mg binds to the phosphorylated NR and inactivates it^{68,69} (Figure 2). Magnesium application is known to inhibit *in vitro* NADH:NR activity but not the methyl viologen activity⁷⁰. Apparently, the electron flow from NADH to the flavin is inhibited in the presence of Mg. The NR-inhibiting protein from spinach has been characterized by Yoshimura *et al.*⁷¹, which is a dimer with sub unit *Mr* of approximately 53 kD.

Divalent cations Ca^{2+} and Mg^{2+} are key regulators of NR activity through their involvement as cofactors for either protein kinases or inhibitor protein. The inhibition of NR activity by these ions as reported in many species appears to be a hysteric property, at least for the enzyme

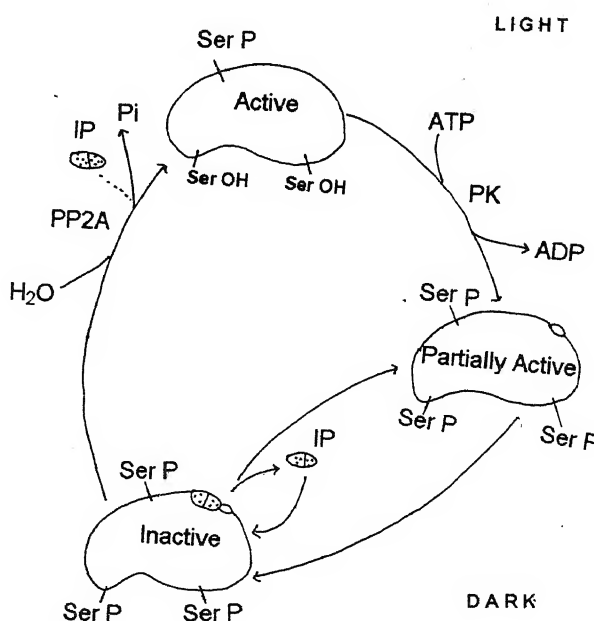


Figure 2. Mechanism of reversible phosphorylation of nitrate reductase during dark-light transition (IP = inhibitor protein; PK = protein kinase; PP 2A = protein phosphatase 2A).

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from spinach leaves⁷². The enzyme extracted from darkened leaves is in low activity or low affinity form, while the one extracted from illuminated leaves is in high activity or in high affinity form^{72,73}. When extracted from darkened leaves, the enzyme which is in low affinity form is inhibited more strongly by Ca^{2+} and Mg^{2+} , than when extracted from illuminated leaves, where the enzyme is in high affinity form. Little inhibition of enzyme is seen when it is converted to high affinity form by preincubation of the low affinity enzyme preparation with the substrate⁷² or with Pi, 5'AMP and FAD⁷³. The low affinity form has a optimum pH of 7.5, while high affinity form has a optimum pH of 7.8 (ref. 74). The conversion of enzyme to a high affinity form in light apparently involves dephosphorylation of the enzyme protein by phosphatases. In *in vivo* experiments, the reactivation of inactivated (darkened plants) enzyme is prevented by okadaic acid, an inhibitor of certain phosphatases⁷⁵. Further, in *in vitro* experiments, the NR that had been inactivated in the presence of NR kinase, ATP-Mg and inhibitor protein could be reactivated either by dephosphorylation with protein phosphatase 2A or by dissociation of inhibitor protein from NR⁶⁹.

Signal transduction

Attempts have been made to trace the path of signal transduction in NR regulation by light at least. Light signals for NR induction is apparently received by the phytochrome, at least in etiolated plants^{36,58,76}. Sopory and his associates have suggested the involvement of Ca^{2+} and phosphoinositide cycle as second messengers in the transduction of signal from active phytochrome to NR gene expression⁷⁷⁻⁷⁹. In fact, Raguram and Sopory⁸⁰ have demonstrated that the involvement of phosphoinositide cycle and protein phosphorylation by protein kinase type enzyme, is a common strategy in signal transduction through phytochromes. Bergarche *et al.*⁸¹ have also demonstrated that the phytochrome was effective only when free Ca^{2+} was available. In addition, a signal originating from chloroplasts is also required for the control of NR expression by light, in green tissues²⁸.

Recently, the presence of a specific receptor for nitrate has been suggested in the plasma membrane, which besides binding with the nitrate may also bind with the respiratory inhibitors azide and cyanide⁸². The significance of the binding of the inhibitors is not understood at the moment.

Concluding remarks

Significant achievements have been made in the study

of the molecular biology of higher plant NR during the last 10 years or so. The story is not complete; the mysteries are absorbing and the prospects of deep inquisitiveness and indulgence are inevitable. The successful creation of transgenics with altered NR structure and function have opened up the possibilities of manipulating this enzyme for qualitative and quantitative improvement of crops and also for the reduction of nitrate contamination of food and feed products. A commercially viable technology for using NR preparation for reduction of nitrate contamination of ground water is to be developed; the probabilities are excellent.

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3' Non-templated 'A' addition by *Taq* DNA polymerase: An advantage in the construction of single and double mutants

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The 3'-end non-templated 'A' addition by *Taq* DNA polymerase has been described as a disadvantage in the generation of site-specific mutants as this 'A'

leads to a unplanned second mutation. Here we demonstrate the utility of this 3' non-templated 'A' addition in the simultaneous construction of single and double mutants of serine hydroxymethyltransferase.

Site-directed mutagenesis (SDM) has been widely used for research in molecular biology and protein engineering. Several methods for SDM using polymerase chain reaction (PCR) have been described¹⁻⁸. Megaprimer method is one of the most rapid and universal, in which one mutagenic primer and two universal flanking primers are required. A possible problem associated with this method is the addition of an adenosine residue at 3'

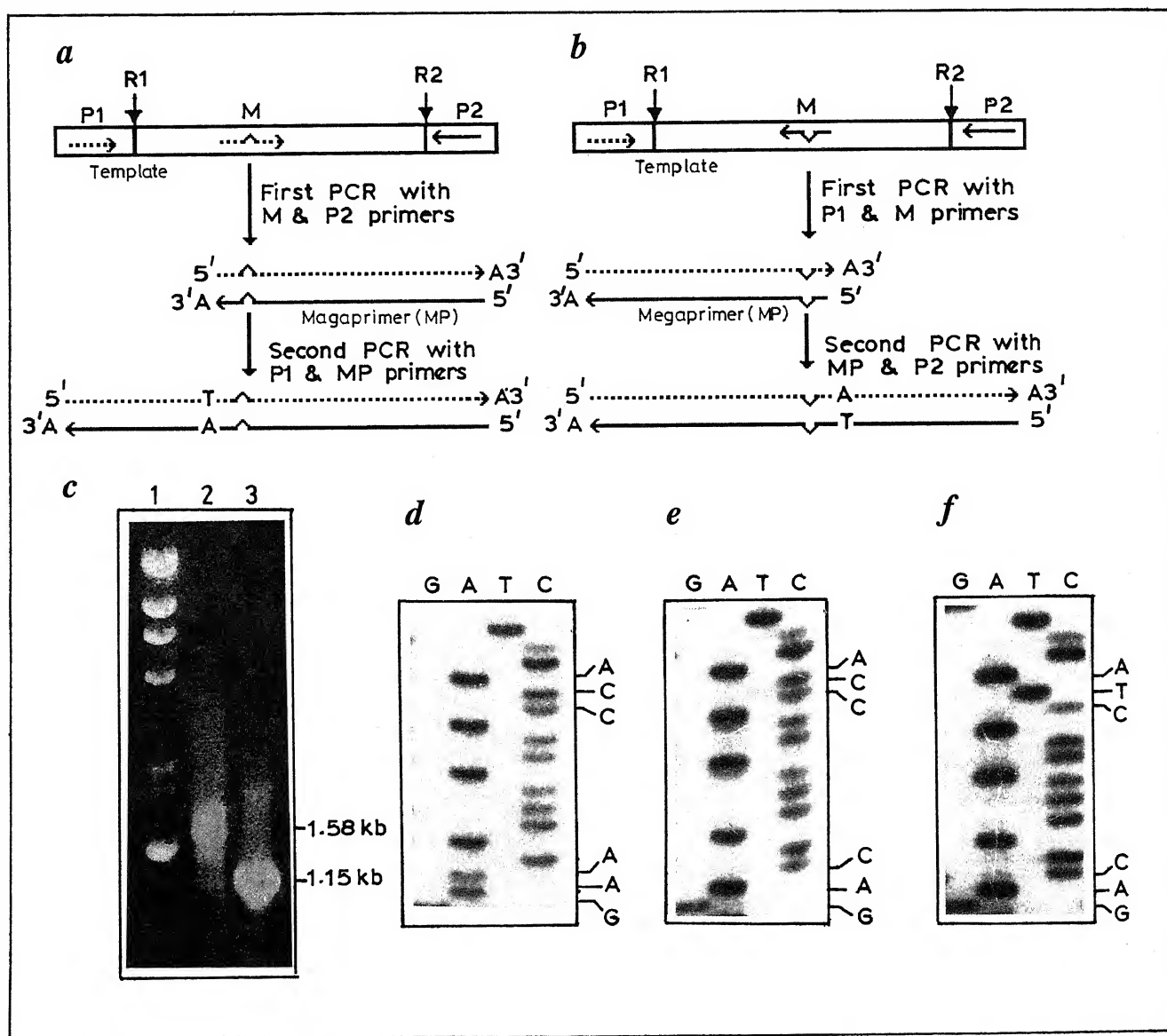


Figure 1 a-c. A megaprimer method for the construction of single and double mutants. *a, b*, Schematic diagram for the construction of single and double mutants. The coding strand of PCR product is shown as dotted lines and non-coding strand in solid lines. Sense and antisense primers are shown as (...) and (←). *c*, Agarose gel with lane 1. Lambda Hind III/pUC 19 Hind I marker, lane 2. Final PCR product with expected size of 1.58 kb and lane 3. First PCR product (megaprimer) with an expected size of 1.15 kb. Part of sequencing gel confirming *d*, wild type SHMT sequence; *e*, single mutant (AAG → CAG); *f*, double mutant (AAG → CAG and ACC → ATC).

end of the PCR product by template independent terminal transferase activity of *Taq* DNA polymerase⁹, resulting in an unplanned mutation. Here we report an advantage of this 3' non-templated 'A' residue addition in the generation of specific double mutants using a single mutagenic primer.

As shown in Figure 1 a, if a sense mutant primer is used it will anneal to the non coding strand and an 'A' residue will be added to the 3' end of the PCR product during first PCR. This addition of an 'A' residue will eventually lead to a 'T' substitution in the coding strand upstream of the desired mutation. In contrast when an antisense primer is used, it will lead to an 'A' substitution in the coding strand downstream of the desired mutation (Figure 1 b). As apparent from the Table 1, by a proper design of the mutant oligos, double mutants can be obtained with varying amino acid substitutions as described in this study.

In our studies aimed at understanding the structure-function relationship of serine hydroxymethyltransferase (SHMT) from sheep liver, we were interested in the construction of single and double mutants of the SHMT gene. The protocol for this purpose involved, the first PCR amplification carried out in a total volume of 100 µl containing 75 pmoles of the 19-mer reverse universal primer (P2) and 20-mer mutagenic primer (M) (H148N mutagenic primer: 5' G GAT GGG GGC AAC CTG CTG A 3' or K257Q mutagenic primer: 5' C ACC ACC CAC CAG ACC CTG C 3') (Bangalore Genei Pvt Ltd., Bangalore, India), 2.5 units of *Taq* DNA polymerase (United States Biochemical, Cleveland, OH, USA), 0.2 mM deoxyribonucleoside triphosphate (dNTP, Amersham International plc, Buckinghamshire, UK), 2 mM MgCl₂, 50 ng of DNA template (pUCSH) and the buffer provided with the enzyme at 1x concentration. The reactions were

amplified in a thermal cycler (COY Tempecycler II Model 110S, COY Laboratory Products Inc., Ann Arbor, MI, USA) with the initial 4 min denaturation at 94°C then for 30 cycles with 1 min denaturation (94°C), 1 min annealing (50°C), 1 min 30 sec elongation (72°C) followed by 10 min elongation at 72°C. Samples were analysed on 1x TAE agarose gel (Figure 1 c). PCR products were purified as described by low-melting temperature-agarose method¹⁰. Purified PCR products were quantitated on agarose gel by ethidium bromide fluorescence. A second PCR was set up using the conditions similar to those described above with 25 pmoles P1 (internal primer for SHMT gene: 5'T ATG GCA GCT CCA GTC AAC 3') (National Biosciences, Plymouth, MN, USA) and 1 µg of purified first PCR product as primers and 50 ng of pETSH DNA as template. We have used different templates in the two PCR amplifications, i.e. pUCSH in the first PCR (1.2 kb SHMT cDNA clone lacking 227 bp at 5' end in pUC19 vector) and pETSH in the second PCR (1.45 kb full length SHMT cDNA clone in pET 3c vector)¹¹ to obtain convenient restriction sites in the final PCR product. However, the same template can be used for both the PCRs. The final PCR product was gel purified, digested with KpnI, BamHI and ligated with KpnI, BamHI digested pUC 19. The ligation mixture was used to transform *Escherichia coli* DH5α cells. The mutations were confirmed by dideoxy double-stranded DNA sequencing¹² using sequence version 2.0 DNA sequencing kit (United States Biochemical).

In the design of a mutagenic primer, a Lys (AAG) residue has been changed to a Gln (CAG). The primer has been designed to begin with a cytosine (C) base corresponding to 3rd base of Thr (ACC) which is 3 amino acids upstream to this Lys in the SHMT gene. Since this is a sense mutant primer, a 'T' will be

Table 1. Anticipated amino acid changes due to 3' non-templated 'A' addition by *Taq* DNA polymerase

Amino acids	Codon	1st base		2nd base		3rd base	
		T	A	T	A	T	A
Phe/Leu	TTN	—	Ile/Met	—	Tyr/*	Phe	Leu
Ser	TCN	—	Thr	Phe/Leu	Tyr/*	—	—
Tyr/*	TAN	—	Asn/Lys	Phe/Leu	—	Tyr	*
Cys/*/Trp	TGN	—	Ser/Arg	Phe/Leu	Tyr/*	Cys	*
Leu	CTN	Phe/Leu	Ile/Met	—	His/Gln	—	—
Pro	CCN	Ser	Thr	Leu	His/Gln	—	—
His/Gln	CAN	Tyr/*	Asn/Lys	Leu	—	His	Gln
Arg	CGN	Cys/*/Trp	Ser/Arg	Leu	His/Gln	—	—
Ile/Met	ATN	Phe/Leu	—	—	Asn/Lys	Ile	Ile
Thr	ACN	Ser	—	Ile/Met	Asn/Lys	—	—
Asn/Lys	AAN	Tyr/*	—	Ile/Met	—	Asn	Lys
Ser/Arg	AGN	Cys/*/Trp	—	Ile/Met	Asn/Lys	Ser	Arg
Val	GTN	Phe/Leu	Ile/Met	—	Asp/Glu	—	—
Ala	GCN	Ser	Thr	Val	Asp/Glu	—	—
Asp/Glu	GAN	Tyr/*	Asn/Lys	Val	—	Asp	Glu
Gly	GGN	Cys/*/Trp	Ser/Arg	Val	Asp/Glu	—	—

The table shows the possible amino acid changes which could arise by 'T' or 'A' substitution in the coding strand at 1st, 2nd or 3rd base of a given amino acid(s). Stop codon is shown as (*), (—) is shown for no change in the amino acid.

substituted for 'C' (i.e. 2nd position in the ACC codon) which will result in the mutation Thr → Ile (ATC), leading to the generation of a double mutant (Lys → Gln and Thr → Ile). Similarly, 'T' to 'C' substitution was observed in the case of *nisA* gene⁶. If the primer starts from the second base of the codon (ACC) then the 'T' to 'A' substitution will take place at first base of the codon resulting in the mutation Thr → Ser (TCC) (see Table 1). Thus by a proper design of mutagenic primers, specific double mutants can be generated. Some DNA molecules will be left without 3' non-templated 'A' addition which would lead to a single mutant (Lys → Gln). We have sequenced 4 clones in which 2 of them are double mutants (Ile-Thr-Thr-His-Gln) and 2 of them are single mutants (Thr-Thr-Thr-His-Gln) (Figure 1 d). Similarly, we have constructed single and double mutants for the SHMT where Pro-Asp-Gly-Gly-His (wild type) had been converted to Pro-Asp-Gly-Gly-Asn (single mutant) and Leu-Asp-Gly-Gly-Asn (double mutant). In this case, we have sequenced 13 clones in which one of them is single and 12 are double mutants. These mutants were expressed and found to be present in the soluble fraction (Jagath-Reddy *et al.* unpublished results). The characterization of these mutant proteins is in progress.

The advantages of this protocol are: (i) To obtain single and double mutants simultaneously. This procedure is useful in the case of highly conserved proteins where there are too many amino acids to be screened from structure/function point of view. (ii) It is more economical as one would need a shorter oligonucleotide (20–25 bp) compared to other methods (30–35 bp) to generate double mutants in which the two mutation sites are separated by 10–15 bp. Although the second mutation in this case is restricted to certain amino acid replacements only, the method is useful for the generation of single and double mutants. The 3' non-templated 'A' addition results only in substitution (confirmed by sequencing more than 20 different mutant clones) and will not result in a frame shift mutation as described⁸. Only two non-specific mutations were noted in more than 8 kb sequence determined for all the mutant clones.

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Direct and indirect somatic embryogenesis in teak (*Tectona grandis* L.)

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Apical and axillary buds from three-year-old teak (*Tectona grandis* L.) were used to initiate the cultures. Callus from apical buds of teak formed globular and heart-shaped somatic embryos on Murashige and Skoog medium supplemented with 6-benzylaminopurine (BAP) (0.1 mg/l) + 1-naphthalene acetic acid (NAA) (0.01 mg/l) and 3% sucrose. However, callus initiated from axillary buds was unable to form somatic embryos on semisolid Murashige and Skoog medium with different combinations of growth regulators. On the other hand, somatic embryos were readily formed from the same callus when transferred to half strength liquid medium containing BAP (0.1 mg/l) + NAA (0.1 mg/l). Somatic embryos were directly formed from axillary buds of teak inoculated in test tubes having filter paper bridges with half strength liquid medium containing BAP (1.0 mg/l) + 2iP (1.5 mg/l).

TEAK (*Tectona grandis* L.) is an important tree known for its high value timber. However, it is slow growing besides the low percentage of seed germination. Tissue culture is a faster method of propagation. The regeneration of teak plantlets by multiple shooting of nodal segments and shoot tips^{1,2} and by organogenesis via callus culture of young and mature leaves³ has already been reported. The present report is on somatic embryogenesis in teak which has not been reported so far.

Apical and axillary buds of three-year-old plants were inoculated on semisolid Murashige and Skoog⁴ (MS) medium (pH 5.7 ± 0.1) and incubated 25 ± 2°C in dark for 72 h and then in 16/8 h photoperiod having 1200 lux light intensity. Callus initiated on MS + 6-benzyl-

aminopurine (BAP) (2 mg/l) after 7 days. Initially callus was hard, compact, white and then slowly turned green. Callus was transferred to MS medium supplemented with various permutations and combinations of BAP, 6-furfuryladenine (KIN), N_6 -(2-isopentyl) adenine (2iP), 1-naphthaleneacetic acid (NAA) in the range of 0.01–2.0 mg/l to induce differentiation.

From callus of apical bud, globular somatic embryos were formed on full strength semisolid MS medium (pH 5.7) supplemented with BAP (0.1 mg/l) + NAA

(0.01 mg/l). They further differentiated into heart-shaped embryos on the same medium (Figure 1 *a,b*). Secondary embryogenesis was also observed (Figure 1 *c*).

From axillary buds both direct and indirect somatic embryogenesis was observed. Since somatic embryogenesis did not occur on full strength semisolid MS medium, the cultures were transferred to liquid, half strength MS medium (pH 5.7). When callus was placed in liquid MS medium supplemented with BAP (0.5 mg/l) + NAA (0.1 mg/l), somatic embryos developed

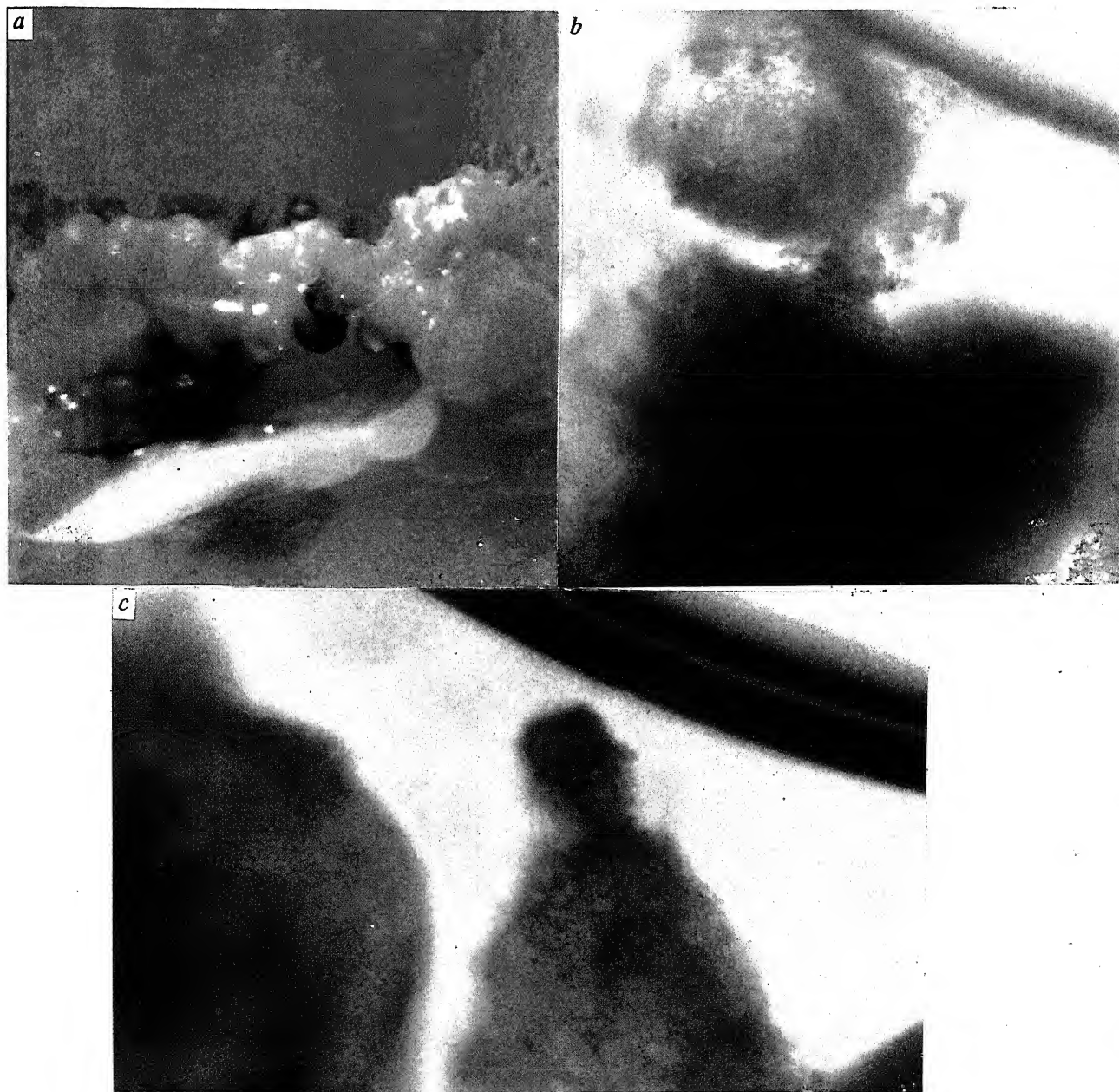


Figure 1 *a–c*. Apical bud cultures of *Tectona grandis* on full strength MS medium. *a*, Indirect somatic embryogenesis; *b*, Globular and heart-shaped embryos; *c*, Formation of secondary embryos.

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from periphery (Figure 2 *b*). Globular and heart-shaped embryos were observed under the microscope after 4–6 weeks (Figure 2 *c,d*).

Somatic embryos were directly formed from approximately 2% of the axillary buds when placed on filter paper bridges in test tubes containing liquid, half strength MS medium supplemented with BAP (1.0 mg/l) + 2iP (1.5 mg/l) (Figure 2 *a*, Table 1).

Direct and indirect somatic embryogenesis from axil-

lary buds occurred on liquid half strength MS medium. Requirement of reduced amount of nutrient (MS with half major salts) for inducing somatic embryogenesis has been reported earlier in ovule culture of *Magnifera indica* by Litz *et al.*⁵ Moreover suitability of liquid medium has also been demonstrated by Raemakers *et al.*⁶ in case of cassava for inducing somatic embryogenesis.

Indirect somatic embryogenesis from the callus derived

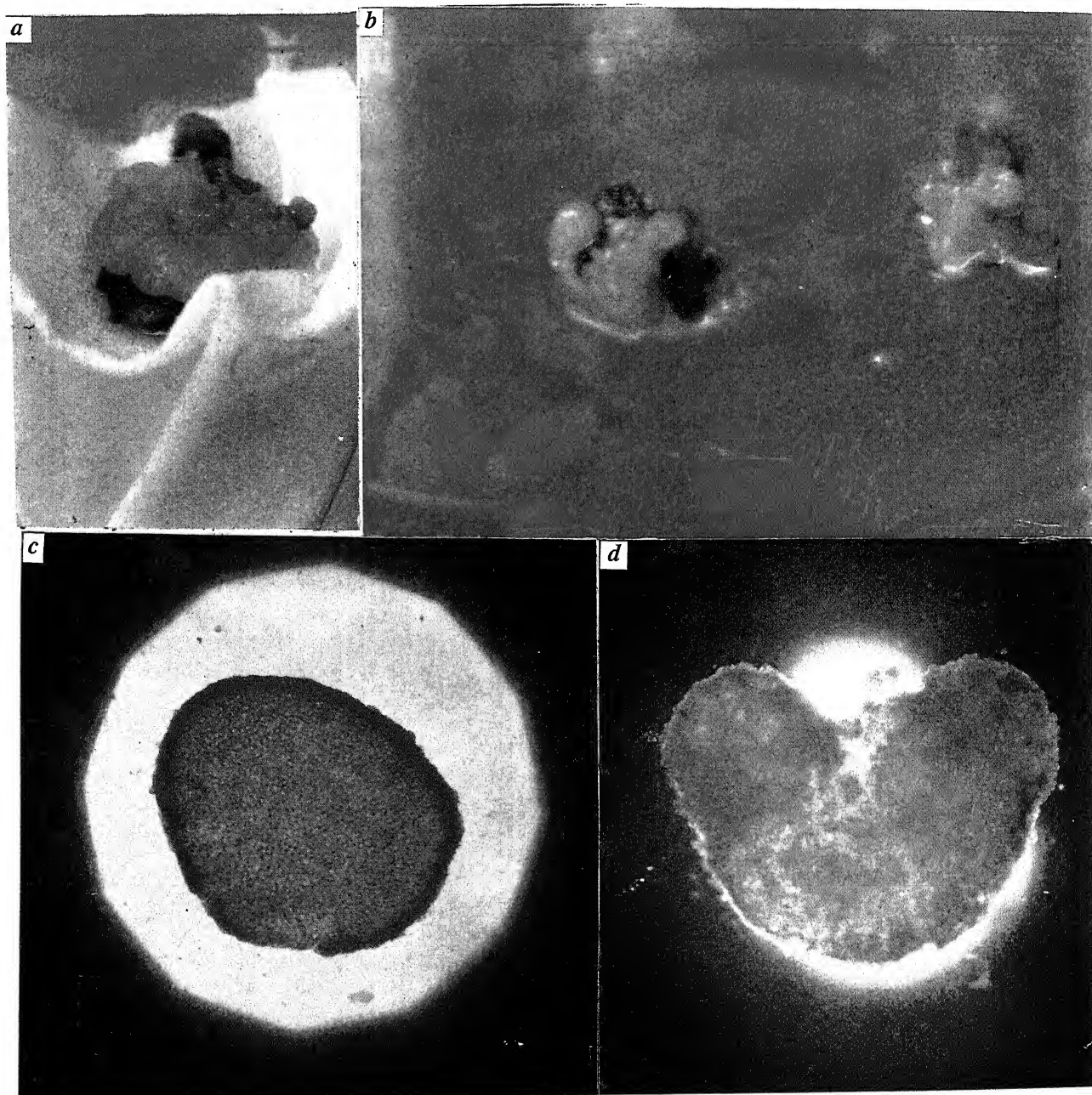


Figure 2 *a-d*. Axillary bud cultures of *Tectona grandis* on half strength MS medium. *a*, Direct somatic embryogenesis; *b*, Indirect somatic embryogenesis; *c*, Globular somatic embryos; *d*, Heart-shaped somatic embryos.

Table 1. Effective media for somatic embryogenesis of teak

Explant	Type of somatic embryogenesis	Medium	Remarks
Callus of apical bud	Indirect	Full strength MS + BAP (0.1 mg/l) + NAA (0.01 mg/l)	Semisolid medium in test tubes
Callus of axillary bud	Indirect	$\frac{1}{2}$ MS + BAP (0.5 mg/l) + NAA (0.1 mg/l)	Liquid medium in test tubes and Erlenmeyer flasks
Axillary bud	Direct	$\frac{1}{2}$ MS + BAP (1.0 mg/l) + 2iP (1.5 mg/l)	Liquid medium in test tubes having paper rafts

from apical buds needed full strength MS medium. Requirement of full strength MS medium was also found in case of apple⁷.

Efforts are on to further differentiate these somatic embryos into plantlets.

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Onset of an arid climate at 3.5 ka in the tropics: Evidence from monsoon upwelling record

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Studies on the variability of Southwest (SW) monsoon strength using the monsoon upwelling indices (fluxes of total planktonic foraminifera and *Globigerina bulloides*) from the western Arabian Sea reveal that the weakening phase of the SW monsoon started from 5 ka (ka = 1000 years). The intensity of monsoon returned to glacial strength at 3.5 ka, coinciding with the onset of arid climate elsewhere in the tropics. The onset of the weak phase of the monsoon and arid climate at 3.5 ka appears to be a primary reason for the decline of Indus Valley Civilization, major change in vegetation along the Western Ghats and decrease of river discharge from all major rivers during that period.

DURING the Northern Hemisphere summer, strong south-westerly monsoon winds blow across the Arabian Sea, causing offshore Ekman transport and intense seasonal upwelling along the Oman and Somalia margins and the Southwest coast of India¹⁻⁴. The upwelling

process brings cold, nutrient-rich waters from a few hundred meters depth to the surface and increases biological productivity in the euphotic zone. During the winter, the Northeast monsoon winds invoke onshore Ekman transport of surface waters, which suppresses upwelling and lowers the productivity along the continental margin of the western Indian Ocean. Thus the south-westerly and north-easterly winds produce a striking seasonal contrast in primary productivity⁵ and biogenic and lithogenic fluxes⁶ in the Arabian Sea. Distinctive plankton faunas and floras thrive in the upwelling waters and are eventually incorporated into the sediments on the sea floor, producing a geological record of upwelling. The sedimentary record in the Arabian Sea is thus linked to the strength of the SW monsoon winds and associated rainfall in southeast Asia. The biogeochemical studies on these sediments therefore provide valuable information on the variability of monsoon upwelling and rainfall in southeast Asian countries over geological time scales. Recently, we have documented the general variability of the SW monsoon for the last 19 ka and its sub-Milankovitch cyclicity^{7,8}. The primary tasks leading to this communication were (i) to find out when the weakening phase of SW monsoon was set in within the late Holocene, and (ii) whether this time coincided with rapid climate shifts elsewhere in the tropics.

RESEARCH COMMUNICATIONS

The upper 7.4 m of the Ocean Drilling Program (ODP) Hole 723A (Figure 1) was sampled at 10 cm interval, which gives an approximate time resolution of 250 years. Radio carbon dates obtained on Accelerated Mass Spectrometry (AMS) were used to establish the chronology (Table 1). High resolution upwelling indices data in conjunction with precise AMS radiocarbon chronology, permit a detailed interpretation of SW monsoon variability and climate change from the tropical Arabian Sea. The details about the AMS dates and upwelling indices are discussed elsewhere⁷.

Several planktonic foraminiferal indices of upwelling were identified in the Arabian Sea⁹⁻¹¹. Fluxes of total planktonic foraminifera and *Globigerina bulloides* were used to measure the upwelling intensity in the western Arabian Sea and in turn the SW monsoon strength in south Asia.

It has been well established that the SW monsoon was weaker during glacial periods and stronger during interglacials¹². Intensification of the SW monsoon started at 12 ka, i.e. after a weak phase during the last glacial period¹³. Within the Holocene, greater values of upwelling indices have been noted between 10 and 6 ka, reflecting

a strong SW monsoon (Figure 2). The values of upwelling indices decrease abruptly at 5 ka, indicating a weakening of the SW monsoon. The lowest upwelling indices in the Holocene occurs between 3.5 and 1.2 ka (Figure 2), suggesting that upwelling and the SW monsoon intensity decreased during this period. At 3.5 ka the upwelling indices exhibit the same values as that at 12 ka, when the monsoon started its intensification after the last glacial period, and from 3.5 ka the upwelling indices decline further. Other evidences such as water levels in Ethiopian lakes¹⁴, palaeohydrological data from western Tibet¹⁵, benthic foraminifera record from eastern Arabian Sea¹⁶, pollen records from Northwest India¹⁷ and $\delta^{13}\text{C}$ values of peat deposits¹⁸ also suggest a weaker SW monsoon during this time. A similar pattern of dry conditions during the late Holocene is also reported from Africa and the regions around Caribbean.

Independent evidences such as pollen studies from the eastern Arabian Sea¹⁹, and down core variations of calcium carbonate in the western Arabian Sea²⁰ and $\delta^{18}\text{O}$ data from Tibet lakes¹⁵ also document the arid climate during this time. This observation is further corroborated by an abrupt change in solar radiation, precipitation,

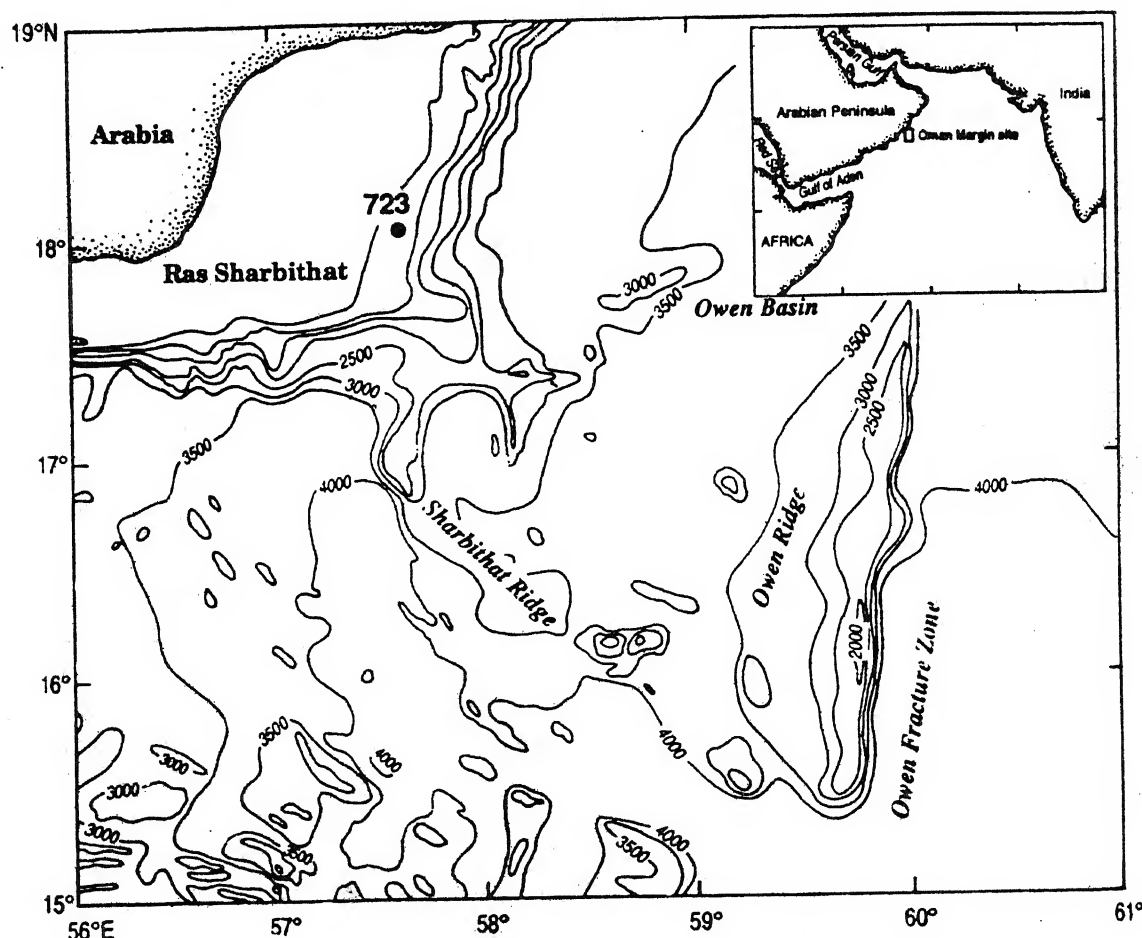


Figure 1. Location of ODP Site 723A, physiography and bathymetry (in metres) of the western Arabian Sea.

Table 1. Radiocarbon ages for ODP Site 723A determined using Accelerator Mass Spectrometer at The Svedberg Laboratory, Uppsala University, Sweden. After Naidu and Malmgren⁸.

Core	Section	Sampling depth (cm)	¹⁴ C ages (years BP)	Error years
1H	1	3	950	± 55
1H	2	160	5,865	± 65
1H	3	290	9,100	± 90
1H	4	520	15,920	± 125
1H	5	740	19,130	± 275

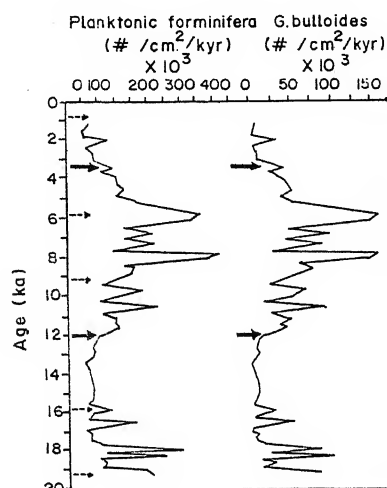


Figure 2. Fluctuations in fluxes of total planktonic foraminiferal shells (>150 μ m fraction) and *Globigerina bulloides*. Ages were based on five AMS ¹⁴C dates at levels marked by dotted arrows. Thick arrows mark the start of monsoon intensification after glaciation (at 12 ka) and the weakening phase of monsoon during late Holocene (at 3.5 ka).

temperature and southwesterly winds at about 3.5 ka in the Arabian Sea²¹. The recent (2–4 ka) aeolian activity in the Thar Desert²² also corresponds with the onset of arid climate at 3.5 ka. Dry episodes including the current drought in the Sahel have been correlated with warming of the surface ocean in the southern hemisphere and the north Indian Ocean, and cooling of the North Atlantic and the North Pacific. The declining strength of the SW monsoon since 3.5 ka can therefore be interpreted as a result of the onset of arid climate in general throughout the tropics and in particular in the Asian tropics.

The onset of arid climate and decline of the SW monsoon at 3.5 ka was resulted in a change in the vegetation along the Western Ghats¹⁹. *Casuarina*, *callitris* shrubs and ferns declined during this period in South Australia²³. The Godavari, Bhima and Krishna rivers originate in the elevated region of the Western Ghats, and their discharge depends on the precipitation driven by the SW monsoon winds. These rivers discharge and lake levels in India, Africa, Australia and China also

dropped during that time. It was previously pointed out that neotectonism was responsible for the drying of the Sarasvati River²⁴. This event could, however, also be attributed to drastic climatic changes at 3.5 ka. It is well established that the down fall of Chalcolithic Cultures (about one million BC) is ascribed to severe droughts in western and central India, similarly the decline of the Indus Valley Civilization around 3.5 ka could be due to the onset of arid climate at 3.5 ka.

An obvious, but provocative question is whether the onset of arid climate at 3.5 ka is traceable in other tropical regions of the world. Evidences such as ¹⁸O/¹⁶O ratios in ostracod shells from Caribbean lakes²⁵ and pollen analysis from south Australia lake²³ have also documented the onset of an arid phase at 3.5 ka. Thus the onset of arid climate at 3.5 ka appears to be a global event, prevailing throughout the late Holocene.

Coherent occurrence of an arid climate at 3.5 ka in the northern hemisphere (present study) and southern hemisphere²⁵, and synchronous dropping and raising of water levels in Australia and China²⁶ indicate that late Holocene climate in the tropics of both hemispheres were in phase.

Two theories, viz. Milankovitch orbital theory²⁷ and deep water formation changes²⁸ have been put forward to explain the causes of glaciation and deglaciation in the Quaternary period. The Milankovitch orbital theory explains the general envelope of past glacial climatic changes, but does not explain either the timing or the amplitude of short-term changes noticed in the present study and in ice core records²⁸. On the other hand, deep water formation changes provide a more satisfactory hypothesis for explaining the ultra fast and abrupt climatic shifts in the ice cores²⁹, and marine sediment records³⁰.

It has been pointed out that thermohaline circulation changes have an influence on the rainfall in the tropics³¹. Deep water formations in the North Atlantic have a profound influence in causing the abrupt climatic shifts at high latitudes³² and probably in low latitudes too. Therefore, I suggest the missing link between the thermohaline circulation and/or deep water formations at high latitudes and monsoon intensity may be initiated to understand the onset of arid climate in the tropics and decrease of monsoon strength.

To conclude, high resolution data on upwelling indices, in conjunction with precise AMS radiocarbon chronology, permit a detailed interpretation of SW monsoon variability and climate change in the tropical Arabian Sea. The decreasing upwelling indices since 5 ka reflect a recent weakening of the SW monsoon, with the weakest phase taking place at 3.5 ka, which coincided with the onset of arid climate in other parts of tropics. The onset of arid climate during that time appears to be the main cause of declining vegetation and river discharge in the Indian subcontinent during that period. The drastic cli-

mate shift at 3.5 ka might be one of the reasons for the decline of Indus Valley Civilization.

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Precambrian–Cambrian boundary microbiota from the Chert Phosphorite Member of Tal Formation in the Korgai Syncline, Lesser Himalaya, India

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Abundant and well preserved organic walled microfossils are recorded from the Chert–Phosphorite Member of the Tal Formation, in the Korgai Syncline, Lesser Himalaya. Two principal categories of microfossils including spheromorph acritarch and small acanthomorph acritarchs are present in the assemblage. Interesting among these two categories are *Leiospheridea* sp., *Micrhystridium regulare*, *M. sp. A*, *M. sp. B*, *M. sp. C*, *M. sp. D* and *Veryhachium* sp. The appearance of large *Micrhystridium* population together with small shelly fauna has been used as a criterion to demarcate the Precambrian–Cambrian boundary in China¹ where these microfossils have been discovered from the black chert in the phosphatic rocks of the lowest Kuanchuanpu Member in Ningqiang of southern Shaanxi.

The Korgai syncline is one of the five major synclines of the Krol belt where the Blaini–Krol–Tal sequence is well exposed. It is located 15 km southeast of Nigalidhar syncline and comprises an area of about 23.50 sq km. Prior to this communication no microbiota was reported from this syncline. The material for this study was collected from a trench located in the NE of Sataun (Figure 1) approximately 4 km from Bargaun on the mule track from Bargaun to Banana village (30° 35' 16" : 77° 40' 44"). Here, the Lower Tal Formation

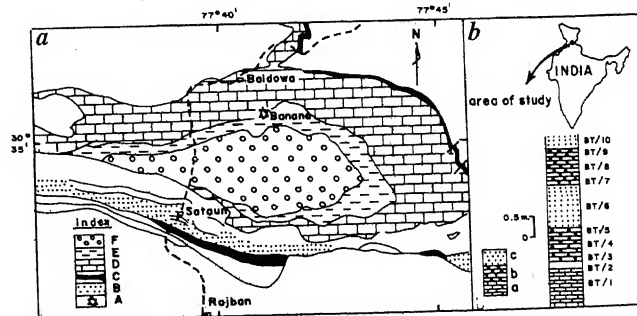


Figure 1a, b. a, Geological map of the area (after Auden, 1934) showing the fossil locality. A = Fossil locality, B = Chandpur and Nagthar formations, C = Blaini Formation, D = Krol Formation, E = Lower Tal Formation, F = Upper Tal Formation; b, Litholog showing sample interval. a = dolomitic limestone, b = cherty phosphorite, c = sandy unit.

consists of thin-bedded cherty phosphorite with intercalated sandy units. Dolomitic limestone of the Krol Formation underlies the cherty shale. The present microfossils are preserved in the phosphatic chert. These fossiliferous cherts are aphanitic and non-laminated. The chert is composed predominantly of cryptocrystalline and microcrystalline quartz with lesser amounts of fibrous calcedony, amorphous organic matter, well preserved microfossils (acritarchs) and minor opaque fine-grained pyrite crystals and orange hematite framboids. All specimens discussed here are observed in petrographic thin sections.

In Lesser Himalaya, spheromorph acritarchs have previously been recorded from the Chert Phosphorite Member of the Tal Formation². Tiwari and Knoll³ described acanthomorphic acritarchs from the stratigraphically lower Infrakrol Formation. However, this is the first report of acanthomorphic acritarchs from the Tal

Formation. The present microbiota is characterized by a unique assemblage. The forms are assigned to *Leiospheridea* sp., *Micrhystridium regulare*, *M. sp. A*, *M. sp. B*, *M. sp. C*, *M. sp. D* and *Veryhachium* sp. (Figure 2) associated with rare filamentous forms. *Micrhystridium* has been known in the lowest Cambrian non-trilobite bearing Meishucun Formation⁴⁻⁸. However, *Veryhachium* sp., which is larger in size in comparison to those species reported from Paleozoic, has not been previously reported from the Precambrian-Cambrian boundary level or the underlying sediments, with the exception of some rare specimens from Proterozoic rocks^{9,10}. This genus is commonly found in the lower Paleozoic which generally is smaller in size.

This communication mainly deals with the discovery of abundant *Micrhystridium* and *Veryhachium* from the Chert Phosphorite Member of the Tal Formation. The occurrence of *Micrhystridium* is very rich in the samples.

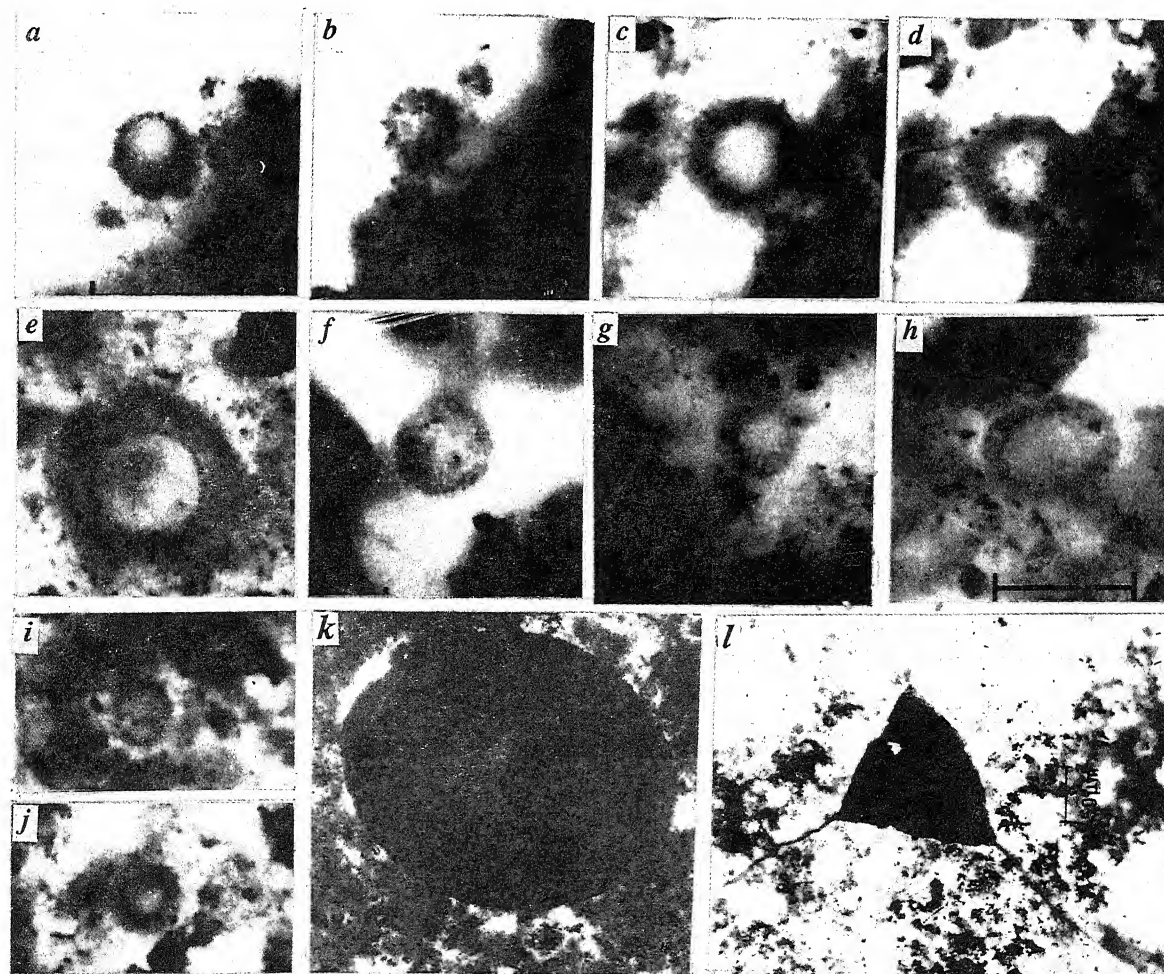


Figure 2 a-l. a, b, *Micrhystridium* sp. A in two different foci, c, d, *M. regulare* in different foci; e, h, *M. sp. B*; f, *M. sp. C*; g, i, j, *M. sp. D*; k, *Leiospheridia* sp.; l, *Veryhachium* sp. Scale bar for Figure h is 10 μ m and for Figure l is 50 μ m. (Scale bar for a-j is shown in l and for k is shown in l).

It is closely comparable to the *M. regulare* reported from southern Shaanxi and also resembles with *Micrhystridium* sp. of Lo described from the Lower Yudoma suite of eastern Siberia¹¹. The presence of *Veryhachium* is unique in the assemblage. Small shelly fossils are not reported from this locality so far, but the acritarchs-yielding level in the Korgai syncline has also yielded SSFs in Mussoorie and Garhwal synclines¹²⁻¹⁵ and also in Nigalidhar Syncline¹⁶. This study shows a close similarity in the microfossil yield of the basal Meishucunian levels in the Lesser Himalaya and the Chinese section. Simple *Micrhystridium* population also occur in the lower Yudoma Formation in Siberia¹¹. Besides, the acritarchs of basal Tal Formation differ greatly from those of Infrakrol Formation³, where large acanthomorphic acritarchs (> 100 µm in diameter) predominate. The presence of smaller acanthomorphic acritarchs in the basal Tal (Cambrian) in comparison to the older Infrakrol Formation (Precambrian) strengthens the concept of decrease in the size of acanthomorphic acritarchs from the Precambrian to the Cambrian¹⁷.

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Potential threat to reservoir fishery by fungi in Kumaun Himalaya, India

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Zoosporic fungi are among the most destructive fish pathogens. Eighteen species belonging to *Achlya*, *Aphanomyces*, *Dictyuchus*, *Saprolegnia* and *Pythium* were isolated from Nanak Sagar, which has witnessed mass mortality of fish fauna during 1991-94. Of these, 8 species were also detected from diseased and moribund fish. Species of the parasites and the hosts demonstrated differential pathogenicity and immunity respectively. *Achlya flagellata* and *Saprolegnia parasitica* appeared virulent, while scaly-fish were more vulnerable to fungal infection than the non-scaly fish. The occurrence of fungal species, zoospore density and severity of mycoses was primarily governed by temperature. A water temperature between 22 and 25°C during spring stimulated fungal activity whereas, at above 28°C it retarded the disease process. All the species, except *A. inflata* and *P. vexans* have been reported virulent fish pathogens elsewhere; this is cause of great concern. By virtue of a wide distribution and potentiality to parasitize eggs, fingerlings and adult fish, these fungi along with anthropogenic pressure are most likely to pose threat to reservoir fishery. Thus, research on integrated management of fish mycoses is warranted.

RESERVOIRS, the artificial ecosystems characterized by the existence of both lentic and lotic components, constitute the prime inland fisheries resource in South and South-east Asia. A major increase in inland fish yield in India is expected from reservoirs because of construction of new dams and the improvement in reservoir fisheries management¹. Beside wide diversity of fish, reservoirs also harbour diverse microorganisms including fungal species which occur as opportunistic pathogens of aquatic organisms, specially of fish^{2,3}. Water molds, the zoosporic fungi, are a constant and ubiquitous component of aquatic environments and a continual challenge to fish⁴⁻⁶. The ability of these fungi, particularly of those belonging to *Achlya*, *Aphanomyces* and *Saprolegnia* to parasitize eggs, fry, fingerlings and adult fish has been well documented⁵⁻¹⁹. As a general rule, infection starts when the host gets injured either mechanically^{9,10} or as a result of infection other than fungal, which often results in epidemic, causing 100% mortality of the infected hosts^{10,13,18}. However, the clinical and pathological investigations have revealed that several members of Saprolegniales often act as primary pathogens^{5,6,11-19}, thereby play a vital role in minimizing the total output

of fish industry either way. In a preliminary study, prevalence of mycoses was observed at a reservoir in Nainital district¹⁹. However, in spite of their great economic value, the zoosporic fungi of the reservoirs are least explored.

Kumaun region abounds in lakes and reservoirs. Behgul, Nanak Sagar, Tumaria and Dhaur harbour a high diversity of fish fauna and are well recognized fish production centres of Government of UP, India²⁰. During a recent exploration (1991–94) of Kumaun Himalaya for water molds, fungal infection was noticed on several species of fish both in lakes¹⁷ and reservoirs^{18,19}. A high prevalence of mycoses on fish species, was, however, observed in Behgul and Nanak Sagar reservoirs¹⁹. Considering the seriousness of the situation, and importance of reservoirs; occurrence, phenology and pathogenic behaviour of water molds to fish and the influence of physicochemical properties of water on the occurrence and infection of zoosporic fungi were investigated at Nanak Sagar.

A large number of individuals of each major contributing fish species were randomly inspected at the fish catching site of Nanak Sagar (29°55'N and 79°40'E; 200 m amsl) spread over 4662 ha in UP hills. Minute signs of disease were observed using a powerful hand lens. Water samples were also collected at a month interval for two years. Certain important characteristics of the water were also estimated following the standard methods of the American Public Health Association²¹.

Symptomatic fry and adult fish were collected in sterile polythene bags, placed in ice in aseptic glass pots and immediately brought to the laboratory. Portions of infected tissue were washed individually with several changes of sterile tap water, and treated with 0.01%

potassium tellurite solution to avoid bacterial contamination. The infected tissues were then aseptically transferred to sterilized petri plates and baited with sterilized hemp seed (*Cannabis sativa* L.) halves. To isolate zoosporic fungi from water, 20 ml of each sample at different dilutions was poured into a sterilized petri plate and baited with different baits⁶. The plates were incubated at 15°C and fungi that colonized were purified, cultured and identified following various monographs^{6,21–22}.

In all, 18 species of zoosporic fungi belonging to 5 genera were isolated (Table 1). *Achlya* and *Saprolegnia* constituted a major proportion, were represented by 8 and 4 species, respectively. Zoospore density of these taxa was recorded in the range of $5\text{--}25 \times 10^3 \text{ L}^{-1}$, which was higher than the species of *Aphanomyces*, *Dictyuchus* and *Pythium* ($2.5\text{--}5 \times 10^3 \text{ L}^{-1}$). These fungi exhibited seasonality of their occurrence, which was closely related with water temperature. Maximum number of species as also high zoospore density of individual species were recorded during February and March (Spring) while a minimum in May and June (Summer). A few species, viz. *Aphanomyces laevis*, *Dictyuchus sterile*, *Saprolegnia ferax*, and sterile isolates of *Achlya* and *Pythium* could resist the summer temperature ($>32^\circ\text{C}$). Majority of these species have shown adaptability to different habitats in the region²⁴.

Of the fungi recovered from water, eight species were also isolated from individuals of 5 fish species exhibiting mycotic symptoms (Table 1). Infection is characterized by depigmentation of body colour, external haemorrhage, descaling, eroded tails and dorsal fins which led to surface swimming and sluggishness and ultimately to death. Colonization of whitish wooly tuft on to infected

Table 1. Occurrence, seasonality and parasitic association of water molds to fish in Nanak Sagar, and elsewhere

Fungal species	Seasonal periodicity	Associated with fish mycoses	
		Nanak Sagar (Reference)	Elsewhere (Reference)
<i>Achlya americana</i>	Spring, rainy		6, 15, 10, 13
<i>A. debaryana</i>	Spring, rainy	18, 19	
<i>A. flagellata</i>	Autumn, spring	19	6, 7, 17
<i>A. inflata</i>	Autumn, winter		
<i>A. klebsiana</i>	Spring–autumn	19	6
<i>A. orion</i>	Rainy, autumn		5, 12, 13
<i>A. prolifera</i>	All season		13
<i>Achlya</i> sp.	All season		6
<i>Aphanomyces laevis</i>	All season	19	13
<i>Aphanomyces</i> sp.	All season		13
<i>Dictyuchus sterile</i>	Spring–rainy		6
<i>Saprolegnia diclina</i>	Autumn–spring	19	6, 17
<i>S. ferax</i>	All season	19	6, 10
<i>S. parasitica</i>	Autumn–spring	19	5, 8, 10, 11, 16, 17
<i>Saprolegnia</i> sp.	Winter–spring		6, 10
<i>Pythium undulatum</i>	Rainy–winter		6, 15
<i>P. vexans</i>	Autumn–spring		
<i>Pythium</i> sp.	All season	19	6

portion is often visible. *Saprolegnia parasitica* was detected from *Mastacembelus armatus*, *Mystus vitatus*, *Tor putitora* and *T. tor*; *S. ferax* from *M. armatus*, *Nandus nandus* and *T. tor*; *A. debaryana* from *M. armatus*, *T. putitora* and *T. tor*; *A. flagellata* was found to be associated with *T. pitutora* and *T. tor*; while *A. klebsiana*, *Pythium* sp. infected *N. nandus* and *T. tor*, respectively. These pathogens caused identical signs in different hosts. However, the disease intensity varied with the host and the pathogen.

The infection began during the first week of February and by the middle of the month, substantial proportion of the fish population got infected. By that time, incidence of mycoses on *M. armatus*, *M. vitatus*, *N. nandus*, *T. pitutora* and *T. tor* was recorded 36.6, 32.0, 29.8, 28.4, 33.6 per cent, respectively. The infection spread rapidly and peaked by the middle of March, decreased thereafter and was minimum in June when the water temperature was high (32°C).

Water temperature between 22 and 25°C, coupled with a high pH (8.6–8.7) and moderate DO (8.5–9.5 mg/l) favoured zoosporogenesis in fungal forms and their subsequent infection on fish. A maximum disease incidence in March coincided with a water temperature of 23°C, a pH of 8.6 and a DO of 9.5 mg/l. Temperature more than 28°C of May through June, suppressed the growth and pathogenicity of the fungal species, resulting in minimum disease incidence of fish¹⁹. Retardation of the pathogenic potentiality of the species at higher water temperature may be due to induction of gammae and oospore formation which cannot infect fish and require a certain period of dormancy^{18,19}. Since July to September is breeding season for the fish, the reservoir is closed for fishing from July to November. Because of this regulation, no data on infection could be available for this period. However, fish eggs which are highly susceptible to fungal infection^{5,6,10,13}, obviously were destroyed during this period. As water molds were abundant during September onwards, their infection on fingerlings and adult fishes is likely. This may be one of the reasons for dwindling fish yield of the reservoir.

Several species of water molds have previously been reported as causal agents of diseases and death in fishes^{4–19}. *A. flagellata* which has long been known as fish pathogen⁷, has recently been reported to be associated with *T. tor* and *Puntius ticto* in temperate lakes¹⁷. Similarly *S. parasitica* parasitized four fish species in Nanak Sagar¹⁹, has also shown potentiality to parasitize several species of fish^{5,8,10,11,16,17}. *A. debaryana* was responsible for epizootic infection on a cat fish¹⁸. Similarly, *A. klebsiana* on *P. conchonious*, *A. laevis* and *S. diclina* on *P. ticto* and *S. ferax* on *Tor tor*⁶ have been reported to be pathogenic.

Moreover, fungal species which were isolated from reservoir but not found associated with fish during the

present investigations, have been reported virulent pathogens of eggs, fry, fingerlings and adults of several fish species elsewhere^{5,6,11}. *A. americana* has been reported as parasite of freshwater fish *Lepomis macrochirus*¹⁰ and several tropical and temperate fish¹⁶. Likewise, *A. orion* has shown its pathogenicity to twelve species of freshwater fish, four belonging to *Puntius*, *Chanda*, *ranga*, *Channa marulion*, *C. punctatus*, *Notopterus notopterus*, *Labeo rohita*, *Colisia lalia* and *Anabas testudineus*^{5,12}. *A. laevis*, *D. sterile* and *P. undulatus*^{6,14} have also been reported pathogenic to cold water fish. Fish eggs are also attacked by these fungi, infected eggs generally do not hatch and are destroyed, thus causing a colossal loss to the fish industry^{5,6,10,13}. A heavily sporulating sterile isolate of *Aphanomyces* has been reported to cause 100% mortality in eggs of *Channa gachua*, while *A. prolifera*, *A. orion* and *Aphanomyces laevis* caused more than 90% egg mortality¹³.

Foregoing discussion reveals that water molds flourishing in Nanak Sagar have wide distribution, and possess great potentiality to parasitize fish⁶. Most of these have a wide host range and any of them can cause substantial loss under congenial conditions. Moreover, a rich mycoflora of parent rivers and within their catchments contribute to the mycoflora of reservoir(s) in terai region. Once established in the reservoir by virtue of parasitism in fish or saprophytic existence, these fungi produce gammae, chlamydospores and oospores that persist for many years and are virtually impossible to eliminate, thus, may pose threat to fish under congenial conditions. Further, the more or less similar mycoflora, Ichthyofauna and physio-chemical properties of water of different reservoirs in terai region has made reservoir fish vulnerable to fungal infection. Under intensive system, such as hatcheries and fish farms mycoses has always been a problem and unless adequate hygienic and husbandry methods are employed, grave losses can result. In some habitats these organisms have been responsible for extinction of some fish species. Himalayan Mahseer, *T. pitutora*, *T. tor* and some other valuable and rare species of Himalayan fish which are disappearing, are prone to fungal infection¹⁹, which beside anthropogenic pressure may contribute to their fast depletion. Unistam²⁵ also reported that water molds were responsible for the extinction of fish species in certain habitats of Australia. Thus, there is an urgent need to undertake prophylactic measures to protect the fingerlings before introducing into the reservoirs. These should be treated with Melachite green, tetracycline, formaline, boric acid and other suggested chemicals in recommended concentrations^{26–28}.

In addition, several species of *Olpidiopsis* are known to parasitize on Saprolegniales. *O. achlyae* on *Achlya* sp., *O. fusiformis* on *A. prolifera*, *O. luxurians* on *A. laevis*, *O. saprolegniae* var. *laevis* on *Saprolegnia* sp.

and *O. pythii* on *Pythium* sp. have been reported from Kumaun²⁴. Thus, hold potential to suppress population of fish pathogens; and intensive research on biological control of fish mycoses is warranted.

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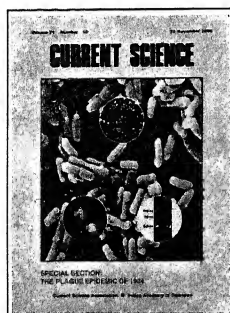
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COVER. Scanning electron micrograph of *Y. pestis* isolated from the sputum of a 2-year-old girl who was admitted to the Civil Hospital, Surat on 1 October, 1994 with acute onset pneumonia and was discharged recovered on 5 October, 1994. *Inset top*: Colony morphology of *Y. pestis* isolated from the sputum of pneumonic patient, on blood agar. *Inset left*: *Y. pestis* isolated from a pneumonic plague case showing fluorescent antibody reaction to F1 antigen. *Inset right*: Plasmid profile of *Y. pestis* isolates analysed by pulsed field gel electrophoresis. See special section.

Indexed in CURRENT CONTENTS/GEOBASE/CHEMICAL ABSTRACTS

The editors thank the Technical Advisory Committee on Plague for help in bringing out the special section.

In this issue

How hot will India be in 2040?

Few topics have generated more heat than the study of global warming. The dispute is not so much about whether it is taking place at all (there is more than adequate evidence to convince almost everyone), but about (a) the relative importance of the contributing factors, and (b) the reliability of the predicted magnitudes of the warming. Opinions really span a wide range. At one extreme, one finds a contemptuous dismissal of the whole global warming research as a gigantic fraud (a dismissal not untinged with envy – few subjects have attracted bigger grants than the study of global climatic change). At the other, there are true believers who claim that unless immediate and drastic steps are taken, vast deserts will be our only legacy to our great grandchildren. In any event, the matters are being taken with enough seriousness at the international level, and the control over discussions is slowly but surely being taken over by administrators, diplomats and politicians from scientists (or is it from scientist-administrators, scientist-diplomats and scientist-politicians?).

The scientists, nevertheless, continue to be busy with the task of refining their models by including more and more factors likely to influence this vastly complex system. Parallely, they have also begun examining the patterns of warming on smaller (and thus perhaps more relevant) scales, and also the finer details of the patterns. One such study, by M. Lal, G. Srinivasan and U. Cubasch (page 746), deals with the situation concerning India – something which would be of more interest to most readers of *Current Science*.

Lal *et al.* have based their investigations on the data generated by

the numerical experiments performed using the European Community Hamburg atmospheric model, coupled to a Large Scale Geostrophic model, which include all such factors as air-sea fluxes of momentum, short and long wave radiation and fresh water. They have used three scenarios – first a control, second one with enhanced CO₂ levels but without sulphate aerosols, and the third which also included sulphate aerosols. Though increased levels of CO₂ cause warming, higher levels of sulphate aerosols are known to cause cooling, thereby moderating the extents to which the temperatures can rise. By comparing the observed rates of warming over the Indian region for the period 1880 to 1989 with those predicted by the models for the three scenarios, the authors demonstrate that only by inclusion of sulphate aerosols can one obtain a satisfactory fit between the data and the model. The observed warming trend of 0.6 degrees per 100 years for the maximum temperature is very well reproduced by the third scenario, which can now be used for predicting the future with a higher degree of confidence.

However, knowing the extent of increase averaged over the entire Indian region will be about as useful as knowing the 'average' depth of a river (for a non-swimmer who wants to cross it). One of the major effects of warming is expected to be on crop productivity, and that varies of course from crop to crop, and therefore from region to region and season to season. With this perspective in mind, Lal *et al.* have investigated the finer details of the pattern of warming. They have primarily looked at the maximum and minimum temperatures, at the annual time scale as well as separately for winter, monsoon, premonsoon and post-monsoon. A curious feature

predicted by them is the reduction in the diurnal temperature range. Looking at the spatial patterns, the model predicts no significant increase for the north-east India by 2040, but suggests a rise of 1.5° along the western margin. Prospects for the other regions can also be seen.

N. V. Joshi

Plague: The aftermath

The plague outbreaks in Maharashtra and Gujarat between August and October 1994, were a sharp reminder of 'India's vulnerability to emerging and re-emerging infectious disease'. The plague episode underscored the inability of the government to react swiftly to unexpected crises and to counter mounting public panic in a calm and effective manner. India's image worldwide was badly affected by the unseemly image of foreign air carriers avoiding a country suddenly host to a medieval scourge. While the government and the media contributed substantially to the autumn madness of 1994, Indian science did not come out covered with glory. The uncertain initial response to the plague outbreak was the result of a continued complacency of the medical establishment to the ever-present threat of communicable disease. For a brief period, the deficiencies of our systems to monitor and unambiguously identify the disease were starkly highlighted. For a while there were only questions. Was it plague after all? What triggered the sudden outbreaks? Why was it bubonic plague in Beed, Maharashtra and pneumonic plague in Surat, Gujarat? Why was the spread of the disease so unlike text-book descrip-

tions of plague epidemics of yore? Did the large-scale consumption of antibiotics (tetracycline) influence the pattern of the epidemic?

Now, two years after the event, *Current Science* carries in a special section (pages 781-808) several technical papers which are the outcome of the work of the Technical Advisory Committee on Plague, constituted by the Government of India. The articles make many key conclusions; the most important being the definitive statement that the organisms isolated from tissue samples of patients in Surat and from rodents and fleas in Surat and Beed are indeed *Yersinia pestis*, the causative agent of plague. Biochemical analysis using PCR methods and ribotyping provide a firm basis for the identification. Some questions still remain. Why was there an extra protein band in the initially observed Surat strains? Was there a direct connection between the bubonic plague of Beed and the pneumonic plague of Surat? The articles attempt to examine the linkages between the ecology of the affected areas and the transmission of the infection. The relationships between the plague episodes and the September 1993 earthquake in Maharashtra and the September 1994 floods in Surat remain unclear. If there is indeed a connection, it is surprising that this disease does not break out more often in a country frequently visited by natural calamities.

The plague committee's report leaves much unsaid. The difficulties

of collection of samples, the problems of inter-laboratory interactions and the valuable time that was lost at the height of the outbreak find little or no mention. By the time the committee was formed, the outbreaks had subsided. The work on identification of the causative organism, therefore, had to depend on samples already collected and stored, with contamination being a major issue (page 782). In the early days, reports of inadequate collaboration and lack of cooperation between investigating laboratories were written about in the popular press. To its credit, the plague committee successfully coordinated a major exercise in scientific investigation spread out over a wide network of laboratories.

What lessons have been learnt? The government has responded by establishing a National Apical Advisory Committee (NAAC) for National Disease Surveillance and Response System. Clearly more than plague looms on the horizon. Malaria, tuberculosis and AIDS are everpresent threats and indeed in 'lay English usage', plague is a term with a much larger definition (page 807). Will the permanent existence of a committee help to combat sudden outbreaks of disease? The dengue fever episode of October 1996 suggests that the lessons of the plague have not really been learnt well. Our public health systems, overburdened far beyond their capacity, are extraordinarily fragile. Our fledgeling biomedical research enterprise is still to come

to a stage where quick, state-of-the-art responses are possible. With limited scientific expertise spread thinly across the country, coordinated efforts have to overcome formidable barriers. Chronic underfunding and a lack of appreciation for the importance of scientific research in most medical institutions have completely damaged the base of medical research in India.

The premier agency, the Indian Council of Medical Research runs a network of laboratories, which surprisingly do not seem to house much of the expertise needed to tackle the problems thrown up by epidemics like the plague.

The plague articles in this issue should stimulate rethinking on our approach to disaster management, particularly in the area of public health. Should we not have contingency plans and pre-arranged networking of laboratories? Should not the Health Ministry be the domain of health professionals and biomedical scientists? Are institutions like the National Institute of Communicable Diseases and ICMR fully equipped for future challenges? We would do well to heed the warning: 'Because of the capacity of microbes to adapt to new circumstances, there will probably be a continuing battle for many years, a subterranean war in which complacency and lack of determination can result in pain and death'. (D. E. Koshland, *Science*, 1992, 257, 1021).

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CORRESPONDENCE

Role of DST

Farooqui *et al.* (*Curr. Sci.*, 1996, 70, 482–483) state at great length that the DST is efficiently encouraging young scientists; within 3–4 months most of the applications are adjudicated. As a contrast, let me record the experience of an old scientist. After working on cephalopods at the Eastern coast for many months at my personal expense and obtaining some results, I submitted an application for a very modest grant for a project to be executed in collaboration with a Central Government organization. In spite of reminders I heard

nothing from DST. After about a year I requested a friend to enquire at Delhi. Several times he did so and the answer was, – ‘Oh, well, we remember that a reply has been despatched but that a copy of it is not available at the moment.’ After another year or so, I despatched a copy of the project to DST explaining everything and requesting them to initiate the project as a new one. This time I received a very prompt reply explaining that (about two years ago) a negative decision had been taken. No referee’s report was included. By that

time the results obtained were in press in the form of a paper and during these two and a half years I have been paying my expenses for food and journey from my pension.

I then applied to the West Bengal DST and a positive decision was communicated to me within two months.

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Long-range forecasts of the total monsoon rainfall of India

The total rainfall over India during the south-west monsoon period, June to September, averaged over a period of more than a hundred years is 85 cm. It is usually called the *normal*. For the last 9 successive years, India had the good fortune of having a normal monsoon, which technically means within plus or minus 10% of 85 cm of rainfall. It is creditable that the India Meteorological Department (IMD) successfully forecasted normal monsoon for all these years. For this purpose, IMD had developed a long-range forecast model called Multiple Power Regression Model, involving 16 global antecedent parameters – 6 Indian and 10 outside India. They are oceanographic, and meteorological – surface and upper air.

Since 1890, on demand from the Government of India, IMD has been supplying long-range forecasts of the total monsoon rainfall over India, with varying degrees of success. Since 1920s they were based on multiple regression models originally developed by Sir Gilbert Walker. Their success has been less than that of the present model.

It may be mentioned that India is ‘top’ in this field.

These long-range monsoon forecasts are for the use of the Ministry of Finance and the Ministry of Agriculture of the Government of India. When a bad monsoon is envisaged, the Finance Ministry has to make financial allotments for meeting scarcity cum famine conditions. Industrial production will be adversely affected due to reduction in hydel power generation and fall in the availability of agricultural raw products. The Ministry of Agriculture has to make arrangements for the dispersal of buffer stocks of food grains to the provinces and districts, for the supply of quick harvesting seeds for a second sowing, etc. An analysis of the past available data shows that a poor monsoon of the kind we had in 1972 or 1986 would result in a loss of around Rs 20,000 crores to the nation (at the present prices). The Finance Ministry therefore needs a long-range forecast of the following monsoon rainfall a month or two ahead of the onset of the monsoon.

In the early days, these long-range monsoon forecasts were not issued to

the public, lest a forecast of poor monsoon should encourage hoarding of food grains by dealers. Later the government decided that these forecasts be published, so that there may be no hoarding at least when a normal or a good monsoon is anticipated.

Of late, a few non-meteorological scientists have severely criticized the IMD saying that such forecasts are of no use for the farmers. Apparently, they do not realize that these forecasts are not meant for the farmers. The IMD gives short-range forecasts for farmers in the *Farmer’s Weather Bulletin*. The meteorological community inside and outside IMD is trying hard to evolve models for providing long-range rainfall forecasts for smaller areas of the size of meteorological sub-divisions and for shorter periods of one month ahead. Let us all wish them success.

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(Edited by A. V. Sankaran)

Oldest emergent continental crust

Even though oldest known Archaean rocks date back to 3.96 billion years, it is not clear when the ancient crust actually emerged out of the primeval oceans that had covered the entire Earth, i.e. when the first continental crust formed and remained stable. This rarity of the early crust (Figure 1) among the continents is attributed by some to the fact that they accumulated slowly over millions of years, while some others believe that most of such crusts that rose above the sea plunged subsequently back into the mantle. However, the search for these early fragments of crust has recently resulted in the discovery of an undisturbed greenstone succession in the Pilbara Craton, Western Australia¹. This strata lying beneath the rocks of the Warawoona Group revealed typical angular unconformity, indicating that they remained stable long enough to be exposed to weathering by wind and water to produce erosion surfaces. Isotopic Pb-U dating of zircon crystals from these rocks indicated their age to

be 3467.6 ± 3.7 million years, half-billion years older than the oldest evidence of continental basement hitherto known (all the greenstone supra-continental volcanic and sedimentary succession deposited over eroded continental basements indicate ages below 3.0 billion years²). The findings have only strengthened the strongly held view that substantial volumes of continental crust existed during the first billion years of Earth's history, though most of them were recycled into the mantle³. This discovery promises to provide a much sought after material – weathered detrital remnants or palaeosols which are strongly believed to lie below the unconformity. These would be very valuable as they could provide useful data about the presence of free oxygen and carbon dioxide in early atmosphere, about the greenhouse effect, and silicate weathering. More importantly, they may provide further information about the beginnings of life on our planet, especially since the oldest fossil cells that

were found more than a decade back were from the very Warawoona Group lying above this unconformity⁴.

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Earthquake precursors

Viable early warning systems for natural calamities such as cyclones and volcanic eruptions are available, but earthquakes have eluded scientific attempts of forecasting. In the recent past three independent groups have published results of their systematic studies on some of the early though (not fully established) indefinite pointers that often precede earthquakes. Two seismologists, Pierre F. Ihmlé and Thomas H. Jordan have noted¹ in 20 cases investigated by them indirect evidences of large energy releases called 'slow earthquakes' (low frequency seismic anomalies) minutes prior to the major event. Their observations are from 19 oceanic events and only one continental event from southern Sudan (Intracontinental transform fault in the East African Rift System). These are important clues mostly obtained from seismic events over the oceanic crust, but to be considered as reliable precursors for predicting earthquakes over the land, systematic monitoring of such early signals in quake-prone regions of the continents must be undertaken.

Sudden chemical changes in subsurface waters have been observed by two teams of Japanese scientists², days before the devastating 7.2 magnitude earthquake of Kobe, Japan, in January 1995. The concentrations of dissolved chloride and sulphate ions in the groundwater below



Figure 1. World map showing areas (striped) more than 2.5 billion years old.

Kobe started increasing steadily five months before the actual earthquake and peaked more than a month after the event. Similarly, concentrations of radon in groundwater increased some four-fold several months before and shot up to ten times just nine days before the January event³. Such co-seismic changes were observed by researchers of Kyoto University also during the 7-8 magnitude events that occurred in 1994. Hydrogeochemical changes of this nature have been noticed earlier in some of the Asian earthquakes also but the data have been mostly of a qualitative nature and no systematic monitoring or quantitative measurements of such precursors were attempted to be useful for early predictions.

Changes in the electromagnetic field (successfully used for prediction originally by Greek physicist Panayiotis Varostos), and emission of high frequency radiowaves have been noted in earthquake-prone areas. Monitoring of these phenomena had enabled prediction of earthquakes in southern California (January 1994) and Kobe in Japan (January 1995) (ref. 4).

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Herbivorous crocodile fossil

Chinese geologists, surveying for petroleum some three decades ago, came across a 120-million-year-old skeleton with unusual features, in the lower Cretaceous of Huei Province of central China, and baffled by the uncommon features of its build, tentatively identified it as a peculiar animal. Recently, the fossil was resurrected from the shelves of Toronto Museum and re-examined¹. It has now been identified as

belonging to crocodile family and has been named *Chimaerasuchus paradoxus*. The skeleton consisting of snout, lower jaw, shoulder girdle, 15 vertebrae, forelegs, hand, pelvis and thigh bones provided a very good reconstruction of the animal – about 3 to 3.5 feet long with forward-pointing nostrils (unlike the normal carnivorous ones with up-pointed nostrils). What was, however, surprising was its multicuspid molar teeth which strongly indicated that the crocodile was herbivorous. Quite unlike the sharp conical teeth of carnivorous crocodiles, *Chimaerasuchus* had relatively flat teeth with distinct cutting edge at the back – features specialized for a fibrous plant diet, and comparable to some of the mammals and mammal-like reptiles.

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Food security in 2020 and beyond – Will the developing countries be dependent on the developed world?

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Food and nutritional security global, regional, national and household is now the focus of concern, debate and conferences. The Food and Agriculture Organization (FAO) will be holding a conference of the Heads of Governments in November 1996 to possibly present the state of food and food security around the world. The heads of governments in their wisdom and commitment to social cause may fix a date by which every human being not only in developing countries, but in the world will have quality food and safe drinking water. The heads of governments had committed to this more than two decades ago. However, today despite enough food production in the world, about 800 million people remain hungry and most of them have a little chance to reach their genetic potential. Food in the past has been a mild weapon in the hands of those who had enough to supply through aid and trade, mostly the latter, but in future it is likely to be a powerful weapon both economically and politically. Therefore, it is time that the developing countries take a serious note of various studies and conferences to be prepared to face the most difficult challenge about to be confronted.

Three major studies or reports have appeared in 1994 and 1995. They are (i) World Agriculture: Towards 2010, An FAO Study¹, (ii) Global Food Projections to 2020 (refs 2, 3): Implications for Investment by the International Food Policy Research Institute under their 2020 Vision Programme, (iii) Full House by Lester Brown and Hal Kane of the Worldwatch Institute projecting the food demand in 2030. Some of these or their preceding studies have also been used as inputs for estimating trade in scenarios of climate change.

The future food demand would be determined by the size of population and purchasing power of the masses, which would be linked with economic growth and employment. The food security in turn would depend upon the balance between the demand and supply

and the latter depends on agriculture because, as of now, there is no such development in sight in science which would replace agriculturally produced articles of food (here agriculture includes both land and water based production systems including forests). There is a fair amount of consensus on projections of population to the year 2010 and 2020 based on UN and World Bank reports. According to these projections, the world population would increase to 8 billion by the year 2020. Of the total increase of 2291 million population in 2020 relative to 1995 population, the developing countries would add 2148 million and the developed countries would add only 143 million. Projections are projections and not predictions (Table 1). How biological system would respond to the changing environment is hard to predict. Nevertheless, we have these projections as the basis of estimating food demand. The projections suggest that five populous countries, China, India, Indonesia, Brazil and Nigeria would have half of the world population by 2020. Most of these countries and many others are improving their economic growth, which would help them improve/change their food consumption pattern. Would the consumption pattern change with increasing income as in developed countries?

Food grains, particularly cereals, are often taken for projecting food demand. This is possibly true of some societies. Therefore, there is an emphasis on projecting the demand for food grains. Also, some of the studies do have projections of animal products, vegetables, fruits, etc. though food grains are generally used as the index. Since we are

dealing with many variables: population, economic growth, climate, technology development, pricing and trade, it is difficult for anyone to make predictions, because a change in any one of the factors could influence the prediction. Therefore, models are used to project demand, supply and trade. The trade for various food commodities is a major outcome of most of the studies. The models do consider the various scenarios of population growth. However, the other inputs are not only based on insufficient data for most countries, but include guesstimates. To quote the methodology of the FAO study 'The results of this research were supplemented by guesstimates'. This is nothing new because guesstimates are liberally used for projecting the impact of climate change on many sectors of the economy. This is partly inevitable because there is a need to project the future of mankind. The fact, however, is that the estimates of demand and supply from different models differ substantially (Tables 2 and 3). For example, one study shows that the former Soviet Union Countries would be surplus in food grains, and yet they had a substantial deficit in 1990. Furthermore, the results show a deficit of 188 million tonnes of food grains by 2020 in developing countries, but the developed countries produce exactly the same surplus for enabling trade between the developing and developed countries as has been shown by the studies of International Food Policy Research Institute (IFPRI). It is projected that in 2020, China would have a deficit of 22.2 million tonnes of cereals, mostly wheat, while India would have a surplus over the demand of 2.2 million tonnes. The

Table 1. Change in population (millions)

	2020	1995	Addition in 25 years
World	8050	5759	2291
Developed countries	1387	1244	143
Developing countries	6663	4515	2148

Table 2. Comparison of cereal net imports by country group (million tons)

	1990*	2000	2010
Developed countries			
Alexandratos	-128.9	n.r.	-157.0
Mitchell and Ingco	-117.4	-142.1	-194.4
Agcaoili and Rosegrant	-112.5	-132.5	-153.0
FAPRI	-14.5	-125.9	n.r.
Developing countries			
Alexandratos	90.0	n.r.	162.0
Mitchell and Ingco	87.0	139.8	210.0
Agcaoili and Rosegrant	82.1	124.2	160.7
FAPRI	91.0	123.7	n.r.
Former CPEs			
Alexandratos	36.4	n.r.	-5.0
Mitchell and Ingco	26.5	2.3	-15.6
Agcaoili and Rosegrant	30.4	8.3	-7.7
FAPRI	23.5	2.2	n.r.

Note: n.r. means not reported.

*Alexandratos' levels are average for 1988-90; Agcaoili and Rosegrant's levels are for 1988.

Source: Nurul Islam 1995.

Table 3. Production of, demand for, and net trade of crops by regions, 1990 and 2020 baseline scenario (see ref. 3)

	World	Developed countries	Developing countries
1990			
Production	1714.7	847.8	866.9
Demand	1714.5	756.6	957.8
Trade	0	91.2	-91.2
2020			
Production	2678.8	1134.5	1544.6
Demand	2678.8	945.5	1722.8
Trade	0	188.2	-188.2

IFPRI study by Rosegrant has used a model where the past trend of yield, the effective producer price, etc. are important components³. A farmer in a subsistence society or in many developing countries grows a crop for his family consumption only, and very few farmers have marketable surplus. Therefore, the effective producer price based on the market price does not play a major role in production by farmers. The past trend in yield cannot always be an indicator for the future. Therefore, the trade models do not capture the realities of production system in developing countries. But according to Brown and Kane⁴ by 2030, the grain deficit in China would be 216 million tonnes and in India would be 45 million tonnes. Thus,

it is difficult to comprehend how such dramatic changes are expected when the population is expected to stabilize by this time. There is either something wrong in models or the inputs used in these models are based on insufficient information. There could as well be a bias towards trade and incapability of the developing countries for meeting their most important needs. The question which should be taken up is whether the present day food importing countries have the natural, human and technological resources to meet their requirements. In the late 50s and early 60s India was described as a nation which would be inflicted by food shortages leading to famines and social disorder. Fortunately, a combination of

Table 4. Predicted net imports of food grains by developing countries

	Year	Million tons
FAO	2010	162.0
IFPRI	2020	188.2
Worldwatch Institute	2030	<433.0

factors in 1966 has made the country self-sufficient. The availability of new technology had a major contribution towards self-sufficiency.

While it is good to state that the developed countries would meet the demand of developing countries, but how many developed countries are today exporting food grains. Most of the export comes from four countries, among them the United States is a major exporter. Would these countries be able to meet the increasing demand for food grains? How much potential they have to produce more in view of the important considerations of sustainability, climate change, declining population on farms (reaching 2 to 3%) and increasing cost of production including the cost of energy.

Several studies in the past made an assessment of production potential of agriculture based on the climate, soil and other natural resources⁵. If these estimates are of any value, then the countries of Africa, Latin America and South Asia are presently producing much lower than their potential. Both technology and investment are the limitations as can be seen by the results of various national and international research centres. For example, the average productivity of Cassava in several countries of Africa ranges from 5 to 12 tons per ha, but experiments at the International Institute of Tropical Agriculture had shown the productivity of 42 tons ha⁻¹ of fresh and 13.0 tons ha⁻¹ of dry Cassava. These examples could be multiplied for the various crops such as rice, wheat, maize, vegetables, etc. If the developing countries have to increase their productivity and production they would have to combine the use of improved varieties with the use of fertilizers, pesticides, water management and post-harvest technology. All these are achievable objectives.

Approach to global food security: The global food security will be

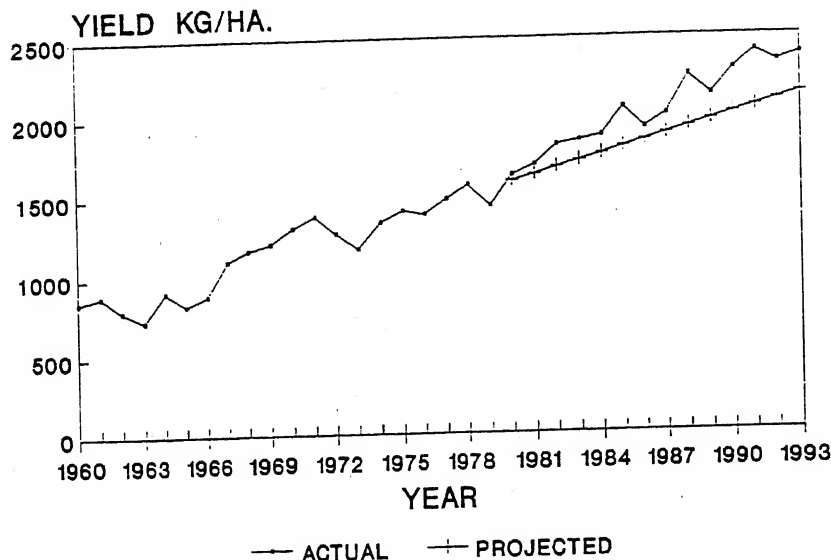


Figure 1. Performance of wheat yield in India.

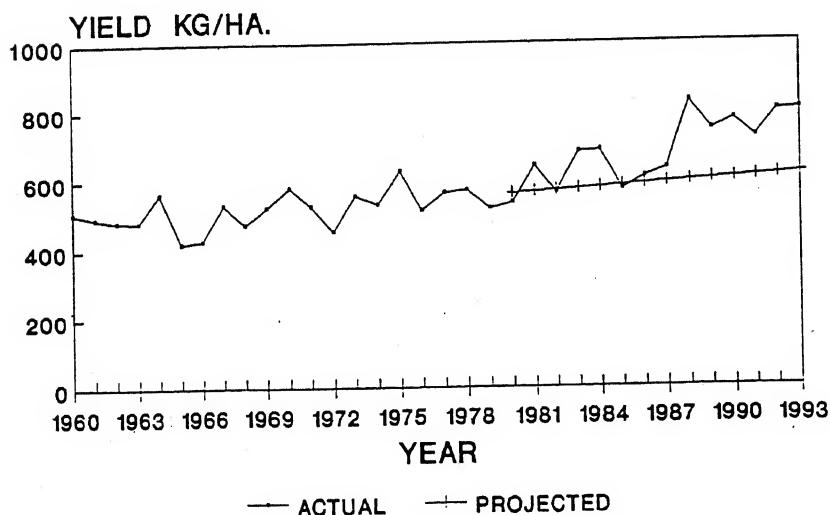


Figure 2. Performance of yield of nine major oilseeds in India.

determined by many factors as listed below:

1. Population growth and its distribution
2. Production of agriculture systems
3. Consumption pattern
4. Economic growth.

There is no doubt that population growth needs to be regulated in developing countries. But is it the only solution as has been advocated⁴? Obviously, the emphasis has to be on the production

of agricultural commodities as well as the consumption pattern preferred by the people. Would the replacement of Cassava and yams produced locally, by imported wheat and rice solve the problem of food security in Africa? This would mean improvement of the local systems of agriculture by introducing modern techniques, but possibly affecting trade in food grain from developed to developing countries. The wheat production in India increased over the past three decades by combining the use of

improved varieties, fertilizers, irrigation, mechanization and improvement of market functioning (Figure 1). As against this the production of oilseeds increased by management of inputs, price and associated aspects (Figure 2). Therefore, there is no reason as to why the developing countries could not acquire food sufficiency at the national or regional basis. The international community should be talking of this rather than telling the developing countries that they have to mainly depend on imports. In conclusion, without going into details the following is required:

- I) The developing countries should not take the projections based on trade models of food security by any agency without scrutinizing inputs and assumptions relevant to their own region and country.
- II) There is a need to assess potential production on the basis of experiments and demonstrations from the research farms, and farmers fields.
- III) There should be an effort by a group of countries in a region to develop a regional food security system.
- IV) The international institutions such as FAO, CGIAR, World Bank and the international community should help developing countries become self-sufficient, preferably through research in the public institutions, and having a fair concern for environment.

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Biosynthesis of selenoproteins

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Selenocysteine is co-translationally incorporated into the nascent polypeptide chain, in both prokaryotes and eukaryotes, through a process directed by a UGA codon. Recently it was shown that special elongation factor, novel tRNA and insertion elements at 3' non-coding region were involved in this biochemical process.

In the recent years, the importance of selenium (Se) as an essential trace element has progressively emerged due to the analysis of selenium deficiency diseases (Keshan disease and Kaschin-Beck disease) and identification of a number of selenoproteins. This element is present within proteins in the form of selenocysteine ($\text{HSeCH}_2\text{CH}(\text{NH}_2)\text{COOH}$), an analogue of cysteine in which the sulphur atom has been replaced by an atom of selenium. In contrast to random incorporation of selenomethionine in the place of methionine in proteins, selenocysteine residues occupy unique positions and are not simply cysteine replacements^{1,2}.

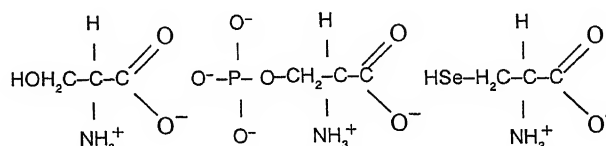
Ever since it was first suggested that Se is an essential component of these proteins, there has been much attention for the mode of incorporation of the Se moiety into the proteins, which occurs by a co-translational process³. With the exception of thiolase and selenoprotein P, glutathione peroxidase, bacterial formate dehydrogenase, Type I iodothyronine deiodinase, protein PA of glycine reductase complex are known to contain selenocysteine, which is also involved in the catalytic function of the above.

Biosynthesis of selenocysteine: the 21st amino acid

Sunde and Evenson⁴ identified serine as the ultimate source of carbon for selenocysteine, and proposed that an additional metabolic step involving the replacement of the phosphate of phosphoserine with selenol (Se-H) group, either while phosphoserine is esterified to the tRNA or perhaps after phosphoserine incorporation into the polypeptide chain, would lead to the synthesis of selenocysteine.

Synthesis of selenocysteine

Serine \longrightarrow Phosphoserine \longrightarrow Selenocysteine



Expansion of genetic code

Chambers and associates⁵ showed selenocysteine, found in the active site of glutathione peroxidase, is encoded by a TGA codon. The existence of a TGA codon in the middle of otherwise an open reading frame was also reported for Type I iodothyronine deiodinase⁶. Berry *et al.*⁶ constructed deletion mutants and frame shift insertions and confirmed that the putative stop (TGA) codon is in-frame, corresponding to selenocysteine. Zinoni *et al.*⁷ also demonstrated an in-frame UGA opal nonsense codon, which directs the incorporation of selenocysteine in the formate dehydrogenase gene. A multiple selenocysteine containing protein, selenoprotein P from rat, was characterized^{8,9} and the corresponding gene¹⁰ sequences were shown to contain ten TGA codons in the open reading frame. Three in-phase TGA codons were also identified in the gene encoding the mitochondrial capsule selenoprotein from mouse sperm¹¹. These results suggest that incorporation of selenocysteine into these proteins can occur by suppression of the UGA codon.

Novel tRNA

The existence of two UGA suppressor tRNAs that can carry phosphoserine in mammalian, avian and xenopus species was demonstrated^{12,13}. Tappel *et al.*^{14,15} identified a tRNA in rat liver that is specific for selenocysteine insertion. Leinfelder *et al.*^{16,17} discovered the *sel C* gene from *E. coli*, encoding an unusual seryl tRNA with UCA at the anticodon loop. This tRNA deviated in several positions from sequences previously considered

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invariant in other tRNA species¹⁸ and is the longest tRNA species with 95 nucleotides, with an acceptor stem of 8 base pairs¹⁸. The novel tRNA accepts L-serine and cotranslationally inserts selenocysteine by recognizing the 'specific' UGA codon. The serine residue, which is attached to unique tRNA^{uca}, is converted to selenocysteine in a reaction dependent on the *selA* and *selD* gene products^{19,20}. This is considered another important pathway for providing additional amino acids like the formylation of methionine attached to the prokaryotic initiator tRNA²¹ and the conversion of glutamate to glutamine during the synthesis of glutamyl tRNA in several organisms²².

Unique translational factor

Forchhammer *et al.*²³ discovered a unique translation factor (SELB), the *selB* gene product, exhibiting extensive homology with sequences of translation initiation factor-2 (IF2) and elongation factor Tu (EF-Tu). Furthermore, purified SELB protein binds guanine nucleotides in a 1:1 ratio and specifically complexes with selenocystinyl tRNA^{uca}. Thus, SELB could be an amino acid-specific elongation factor, replacing EF-Tu in a special translation step. A relevant question for understanding translation and the evolution of the genetic code is, how the translational machinery can cope with the situation that one and the same triplet codon can signal either chain termination or selenocysteine insertion?

Context effects

Bock *et al.*^{24,25} made deletions in *E. coli* formate dehydrogenase gene and fused in-frame to the *lacZ* reporter gene. Their analysis revealed that a sequence of 40 bases in the mRNA at the 3' side of the UGA codon can be folded into a putative hair pin structure, and is required for selenocysteine incorporation. Mutagenesis of the hair pin structure showed that sequences of the loop region are particularly important and serve as a recognition element for special elongation factor, directing the selenocysteine-inserting tRNA species to decode a particular UGA.

Similar structures are conserved in the 3' untranslated regions in many, albeit not all, selenoprotein mRNAs²⁶. Shen *et al.*²⁷ abolished selenocysteine insertion to glutathione peroxidase by deleting four nucleotides at the 3' untranslated region of glutathione peroxidase gene and suggested hairpin-forming region was essential for translational insertion of selenocysteine at a UGA codon. Similar functional elements in the 3' untranslated region of the rat selenoprotein P mRNA, with predicted stem-loops were observed^{28,29}. Footprinting experiments

showed that a special elongation factor, SELB binds specially at the loop region of the hairpin structure³⁰. The targeted insertion of selenocysteine is accomplished by the recognition and binding of ternary complex (special elongation factor and charged novel tRNA) to the mRNA insertion elements in the immediate neighbourhood of in-frame UGA codon^{30,31}. Removal of insertion elements from the 3' end leads to termination of protein at the UGA codon.

Expression of gene during selenium deficiency

Western blot analysis for the detection of immunoreactive protein in liver cytosol obtained from rats fed on selenium deficient diets revealed that glutathione peroxidase protein was not expressed³². Northern blot analysis confirmed three-fold induction of mRNA coding for glutathione peroxidase in livers of selenium-deficient rats^{33,34}. These results indicate that mRNA is synthesized and accumulated to elevated levels in tissues of rats fed on selenium-deficient diets and that selenium status appears to regulate the translation of selenoproteins.

Conclusion

As we trace the pathway of the development of our understanding of biosynthesis of selenoproteins, several interesting features emerge – 'extra' coding capacity by UGA codon³, diversified tRNA¹⁸, existence of specific elongation factor²³, insertion elements in non-coding region^{29,30} and elucidation of selenocysteine as being the 21st amino acid³⁵ – making the protein-synthesizing system a more flexible one. What more surprises could the selenoprotein molecule hold?

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Hormones, cytoskeletal proteins and cell shape

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Recently attention has been focussed on the molecular mechanisms involved in cell shape changes brought about by chemical signals through cell surface proteins. The shape of any given cell type is maintained by various components of the cytoskeleton (microfilaments, intermediate filaments and microtubules), the extracellular matrix, the plasma membrane and associated proteins. Hormones and growth factors regulate the expression and post-translational modifications of these proteins which in turn depend on the phenotype of the cell. Action of hormones and growth factors on cells is also dependent on various factors such as phase and age of the cell, ion gradient and presence or absence of specific receptors at the right sites. The cause and effect in regulation of cell shape is not yet very clear.

THE change in cell shape can be considered as a primary adaptation of the cell to suit altered physiological de-

mands. Cell shape changes are manifestations in many of the cellular events such as division, differentiation, transformation and death. A variety of observations suggest that cell shape changes exert specific effects on gene expression¹. The exact mechanisms by which changes in cell shape affect the pattern of gene expression are not yet very clear but the cytoskeleton seems to play a key role in this process^{2–4}. To understand functional and spatial relationship of the cell in a given tissue, it is necessary to know how the local microenvironment acts on the cell to regulate its phenotype. Cell phenotype is controlled by restructuring the cytoskeletal framework which in turn depends on interactions with neighbouring cells and extracellular matrix (ECM)^{5–8}. A host of extracellular and intracellular proteins are involved in shaping the cellular architecture. The extracellular matrix proteins such as collagen, fibronectin, laminin, proteoglycans and fibrinogens⁹, the transmembrane proteins such as integrins, cadherins¹⁰, the cyto-

plasmic cytoskeletal proteins, microfilaments (MFs), intermediate filaments (IFs) and in some instances microtubules (MTs) which are sites for immobilizing signaling molecules, regulatory enzymes and metabolic substrates transduce the message to the nucleus¹¹. Various factors namely hormones, growth factors (GFs), tumour promoters are known to regulate the expression and/or the activity of many of the above mentioned proteins. These hormonally regulated proteins modulate cytoarchitecture of the target cells¹²⁻¹⁵. Altering the cytoskeletal structure may in turn change the availability of regulatory and catalytic sites of key signal transducing molecules.

During the various physiological processes, the cells undergoing altered cell morphology receive signals from the exogenous chemical factors and through different cell-signalling molecules present at the membrane and in the cytoplasm the information is transmitted to the nucleus where in coordination with internal signals, induction of specific gene expression takes place^{16,17}.

The study of mechanisms involved in the regulation of cell shape change and gene expression by various factors is an active field of research. Hormones and GFs are known to affect the expression, synthesis and post-translational modifications of cytoskeletal proteins. Plasma membrane fluidity is also known to change due to hormone treatment and change in fluidity is known to affect surface projections and regions of attachment of cytoskeletal elements to plasma membrane^{18,19}. Modulation of cytoskeletal proteins and plasma membrane constituents by hormones is responsible for change in cell shape followed by altered gene expression. In attempting to understand the genomic effect brought about by cytoskeletal proteins, Puck and Krystosek²⁰ have shown that during reverse transformation there is an increased sensitivity of DNA to hydrolysis by DNase I in the presence of the factors such as cyclic AMP, retinoic acid and nerve growth factor (NGF). They observed in the ovary derived normal fibroblasts and cyclic adenosine monophosphate (cAMP) reverse transformed chinese hamster ovary cells (CHO-K1) a clear region of DNase I sensitive DNA around the nuclear periphery. In malignant cells specific differentiation genes were sequestered and therefore could not function and exposed regions were not observed. It was also found that the cytoskeletal disorganizing agents such as colcemid and cytochalasin B prevented the basic feature of reverse transformation in enhanced genome exposure, that is closely linked to the transcriptional activation of certain genes. Cytoskeletal proteins such as the IFs and nuclear lamins seem to be actively involved in increased genome exposure, bringing about altered cell morphology.

The various factors (such as hormones) affecting cytoskeletal organization and the non-genomic and genomic pathways involved in bringing about alterations in cell shape are briefly described in this review.

Regulators of cell shape

An analysis of mechanism of shape determination reveals that the ECM and cytoskeleton are the principal determinants of cell shape^{5,7,8,21}. The ECM by itself can influence the shape of a cell. Cells grown on ECM substrate have cell shapes, proliferation rates and responses to GFs which differ from cells grown on plastic or glass²². For instance, granulosa cells cultured on plastic become flattened in shape and develop stress fibres but when grown on ECM the cells become round and closely resemble their counterparts *in vivo*¹². Similarly mouse mammary epithelial cells become cuboidal and increase milk protein synthesis when grown on floating collagen gels²³ as compared to cells on plastic or collagen coated plastic²⁴. It is now generally accepted that there is a physical link between the extracellular matrix and the cytoskeleton. The link is established with the interactions of the ECM via their cell surface receptors to the actin cytoskeleton. Many of the receptors for ECM proteins such as collagen^{25,26}, fibronectin and their receptors integrins¹⁰ and laminin²⁷ have been isolated and these receptors are linked to the actin cytoskeleton within the cell. Fibronectin and collagen are known to be involved in hormone-induced responses^{28,29}.

Changes in cell shape also involve reorganization of the cytoskeletal elements – MFs, MTS and IFs. The use of inhibitors of cytoskeletal protein polymerization such as cytochalasins, colcemid, vinblastine and nocodazole has proved beyond doubt that MFs and MTs are actively involved in change in cell morphology³⁰⁻³³. Many hormones, growth factors and tumour promoters are known to alter cell morphology by affecting the assembly/disassembly of cytoskeletal proteins^{12,14,32,34}.

The ability of actin to polymerize/depolymerize enables the cell to rearrange the MF organization. Anchorage-dependent cells adhere tightly to the underlying substratum through focal adhesions. In many cultured cells, large bundles of MFs are prominent at focal adhesion points³⁵. Many characteristics reveal that they are structurally and functionally equivalent to adhesions made by the cell to ECM *in vivo*³⁶. The actin cytoskeleton is linked to plasma membrane proteins, adhesion plaque proteins and to cytoskeletal proteins (Figure 1). Factors affecting actin or actin-associated proteins can also bring about change in organization of the cell.

The rounding of granulosa cells on treatment with follicle stimulating hormone (FSH) was reported to be associated with down-regulation of synthesis of adherens junction proteins, namely α -actinin, actin and vinculin¹². Lomri and Marie^{14,34} found that parathyroid hormone (PTH) elicited an increase in synthesis of actin in cultured mouse osteoblastic cells. Actin assembly/disassembly was found to be regulated by the expression of actin and vinculin by a feedback loop in 3T3 and HeLa cell lines. In cells which showed elevated

levels of depolymerized actin, the actin mRNA was found to be reduced³⁷.

The IFs are also known to be involved in cell shape changes. For example, upon stimulation of MCF-7 cells (human breast cancer cell line) by estradiol an increase in the keratin filament network results into cytoplasmic ridges on the cell surface^{38,39}. In primary cultures of rat vaginal epithelial cells (VEC) on addition of estradiol, Vijaysaradhi *et al.*⁴⁰ found long microridges on the cell surface which are characteristic of cornified cells indicating increased keratin network just below the plasma membrane. Phorbol esters known to mimic hormone action also bring about cell shape changes during transformation. A reorganization of cytokeratins along with disruption of junctional complexes was observed when Madin-Darby bovine kidney (MDBK) cells were treated with tetradecanoyl phorbolacetate (TPA)⁴¹. Microtubular rearrangement was found in rat pheochromocytoma cells upon activation by NGF⁴². Tubulin synthesis increased by two-fold in response to NGF whereas the synthesis of microtubule associated

protein (MAP) and Tau increased 20-fold. Increased synthesis of tubulin and other MAP proteins are associated with changes in organization of MTs⁴³. These studies suggest a link between changes in cell growth, differentiation, configuration and cytoskeletal protein synthesis.

Regulation of cytoskeleton and associated proteins

Extracellular and membrane proteins

The cytoskeletal elements are in close association and are capable of interacting with each other and with the transmembrane proteins. The ECM transduces a series of signals to the nucleus through cytoskeletal elements. This mechanism of signal transduction may be the result of mechanochemical and/or biochemical processes. In the mechanochemical process the cytoskeleton may regulate gene expression by interacting with the nuclear matrix which may lead to physical expansion of nuclear pores, thereby increasing the rate of nuclear transport in

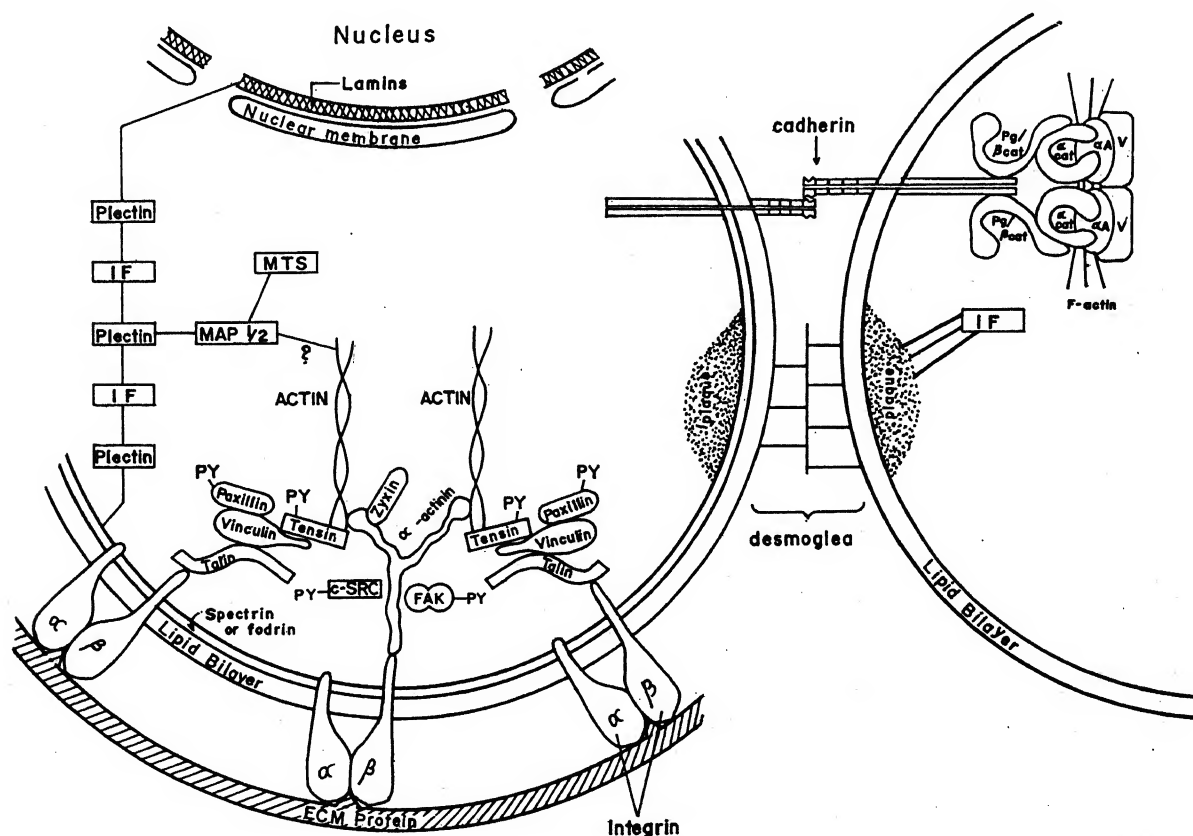


Figure 1. Schematic diagram of two cells depicting the association between cytoskeleton and ECM at focal adhesions; the desmosomes which form another link between two adjacent cells are connected to the cytokeratin IF. Integrins, the receptors for ECM proteins such as fibronectin are connected to actin filaments through two distinct linkages. Talin–vinculin–tensin form one bridge while α -actinin forms another. The actin filaments appear to be connected to IFs and MTs via the MAPs and IFAPs (plectin). The cadherins which connect two cells are linked to actin via the β -catenin/plakoglobin- α -catenin and α -actinin. FAK, focal adhesion kinase; PY, phosphotyrosine; IF, intermediate filament; MT, microtubule; MAP, microtubule associated proteins; Pg, Plakoglobin; β cat, β -catenin; α cat, α -catenin; α A, α -actinin; V, vinculin.

spreading cells⁴⁴. Signals from the ECM reach the nucleus by a series of enzymatic events at the plasma membrane level which in turn involve cytoskeletal elements to convey the message to the nucleus. Among the prominent plasma membrane proteins associated with actin are the cadherins and integrins. Cadherins, calcium dependent-cell adhesion molecules, are transmembrane proteins that interact with cytoplasmic proteins called catenins (α and β -catenins). Catenins link cadherins to the actin cytoskeleton⁴⁵ (for more details, see the legend of Figure 1). It is evidenced by the fact that the cells which express cadherins but lack catenins are of non-adherent type^{46,47}. Cadherins through interactions with catenins form adherens type cell junctions in epithelial cells^{48,49}. β -catenin or plakoglobin constitutes a bridge between cadherins and N-terminal domain of α -catenin. α -catenin then connects this membrane-associated complex with actin cytoskeleton either directly or indirectly via α -actinin⁵⁰. It is suggested that the control of equilibrium levels between free and bound pools of catenins could play a role in regulating cellular responses to extra-cellular signals for cell-cell adhesion or cell proliferation. It seems the phosphorylation and dephosphorylation of catenins regulate the ratio of free and actin bound cadherin-catenin complexes, thereby regulating the adhesive forces of cadherins⁵¹. Desmosomal cadherins, the desmocollin and desmoglein are connected to the IFs via desmoplakin and band 6 protein in contrast to other cadherins which are connected to actin filaments directly⁵⁰.

Integrins which act as receptors for many extracellular proteins, such as collagens, laminin, entactin, fibronectin, vitronectin and fibrinogen, are associated with the actin cytoskeleton inside the cell⁵². Integrins appear to be connected to actin filaments through two distinct linkages. Talin, vinculin and the actin-capping protein tensin form one bridge, while the actin filament cross-linking protein α -actinin forms another (Figure 1; see refs 52, 53). The integrins are essential for cell adhesion both *in vitro* and *in vivo*^{54,55}. Integrins have a role as signalling receptors where they effect the release of second messengers due to hydrolysis of phosphatidylinositol 4,5 biphosphate (PIP₂) which requires both adhesion of cells to ECM and binding of platelet derived growth factor (PDGF) to its receptors. Clustering of integrins in focal adhesions triggers activation of a phosphatidylinositol phosphate (PIP)-5-kinase that catalyses phosphorylation of PIP to PIP₂ and clustering of PDGF receptors by PDGF triggers the activation of phospholipase C (PLC). When cells receive both these stimuli, significant breakdown of PIP₂ occurs, causing release of second messengers such as inositol triphosphate (IP₃) and diacylglycerol (DAG) (Figure 2)^{52,56}. Streuli *et al.*⁵⁷ demonstrated that mouse mammary epithelial cells can direct β -casein gene expression in the presence of prolactin (PRL) and laminin, a compo-

nent of the basement membrane. A cooperative signalling through integrins and PRL receptor is necessary for the differentiated phenotype. These examples demonstrate that adhesion to ECM proteins enhances responsiveness of certain cells to hormones.

Adhesion plaque proteins

The expression and post-translational modifications of adhesion plaque proteins such as vinculin and α -actinin regulate cell adhesiveness. Decreased expression of vinculin or its phosphorylation may result in an inefficient assembly of adhesion plaque proteins leading to decreased adhesion^{12,58}. On the other hand, increased synthesis of vinculin may facilitate more efficient recruitment and assembly of adherens junction proteins leading to increased tightness of adhesion and decreased motility of the cell⁵⁹. α -actinin known to cross-link MFs is also involved in binding actin filaments to the membrane. Increased level of α -actinin at the junctions leads to more stable MF-membrane interaction and hence increased tightness of adhesion⁶⁰.

Many extracellular factors induce phosphorylation of focal adhesion proteins at tyrosine residues^{61,62}. For example, binding of cells to fibronectin and stimulation by neuropeptides results in tyrosine kinase p125^{FAK} (focal adhesion kinase) phosphorylation⁶³. Substrates for activated p125^{FAK} include paxillin and tensin, the components of focal adhesion plaques. Overexpression of FAK-C-terminal domain suggests that FAK may regulate focal adhesion assembly via protein-protein interactions^{64,65}.

Microfilament proteins

Cell surface appendages like microvilli and microridges are supported mainly by MF proteins such as actin. Actin MFs are prominent at focal adhesions and are linked to integrins in the plasma membrane via talin, vinculin, tensin, α -actinin and zyxin^{52,66}. Structural changes in actin and plasma membrane components during cell division, transformation and cell death bring changes in cell shape. Luciano *et al.*⁶⁷ have shown that during the apoptotic programme of enterocytes, the MFs supporting the microvilli at the terminal web region and zonula adherens are withdrawn due to depolymerization of actin and therefore bands of microvilli are detached from main cell body and cell attains a rounded morphology.

During the G-1 phase of the cell cycle, certain types of cells in culture show large number of microvilli, blebs and ruffles which diminish on the onset of S phase and finally the cells become relatively smooth. The microvilli increase in number during G2 phase as cells thicken in anticipation of rounding up for mitosis. MFs reorganize as cells round up for mitosis and provide

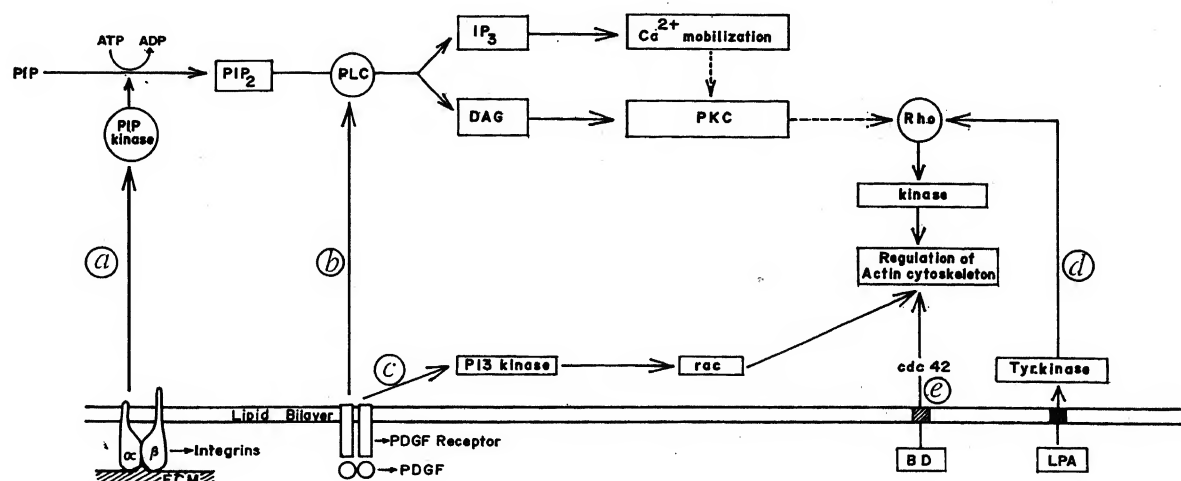


Figure 2. The various signal transduction pathways regulating actin cytoskeleton in Swiss 3T3 fibroblast cells. The ligand-receptor complex transduces the message to the rho family of small GTP-binding proteins via specific kinases. Some of these kinases are phosphatidylinositol 3-kinase (PI3K), protein kinase C (PKC) and tyrosine kinase. *a*, Adhesion of cells to ECM via the clustering of integrins triggers PIP kinase resulting in increased levels of PIP₂; *b*, Binding of PGDF to its receptor activates PLC resulting in calcium mobilization and PKC activation. Rho may be present downstream of PKC regulating actin polymerization; *c*, PGDF and insulin stimulate rac via PI3K which affects actin polymerization at the region of membrane ruffles; *d*, Lysophosphatidic acid (LPA) regulates rho via tyrosine kinase which affects actin polymerization at the region of stress fibres; *e*, Bradykinin (BD) acting via cdc42 affects actin in filopodia⁷⁹. Upstream of each rho family member guanine nucleotide exchange factors are needed which keep them in active form and the GTPase activating proteins (GAP) negatively regulate their activity.

contractile force during cytokinesis⁶⁸. So also mitogenic hormones such as estradiol prepare the cells to divide by causing the cells to shed off microvilli and cells round off in the basal layer of vaginal epithelium of rat^{4,69}. During the process of differentiation, cuboidal basal cells transform to flattened cells with an increase in fluidity of the plasma membrane⁷⁰. During both the processes, cell division and cell death, cell surface appendages are withdrawn, the contact points between plasma membrane and actin fibres are reduced and MF network collapses⁷¹. The levels of cholesterol and proteins on the membranes are reduced and membrane becomes more fluid⁷².

In *in vitro* experiments, stimulation of quiescent serum starved Swiss 3T3 fibroblast cells by various GFs, bradykinin or lysophosphatidic acid result in rearrangement of F-actin⁷³. Such kind of cellular responses at the plasma membrane seem to constitute distinct signalling pathways controlled by Rho family of small GTP-binding proteins such as rho, rac or cdc42 inducing formation of stress fibres, membrane ruffles or filopodia (Figure 2)⁷⁴. There is an evidence that stimulation of rac by PDGF and insulin is mediated by PI3 kinase⁷⁵. A tyrosine kinase appears to be required downstream of rho since rho-mediated induction of stress fibre formation in Swiss 3T3 cells is inhibited by tyrosine kinase inhibitor genistein⁷⁶⁻⁷⁹. Rho is also found to regulate enzymes such as phosphoinositide-3-kinase and phos-

phatidyl inositol-4-phosphate-5 kinase. Hence rho is involved in regulation of actin cytoskeleton through the formation of phospholipid intermediates⁸⁰.

Thus several signals induced by growth factors, hormones and tumour promoters have been implicated in regulating actin cytoskeleton. The classic second messengers IP₃ and DAG produced by hydrolysis of PIP₂ seem to play a role in the signalling cascade. DAG stimulates protein kinase C (PKC) and pharmacological activators of PKC, phorbol esters stimulate actin reorganization in fibroblasts^{77,81}. One of the isoforms of PKC⁸² and various other kinases of the src family and PLC have also been localized to adherens junctions. Events like PKC activation by hormones or GFs involve reorganization of vinculin, depolymerization of actin and phosphorylation of certain proteins that are important for stabilization of the MF-membrane interaction (Figure 2). Woods *et al.*⁸³ have found that PKC activation may play an important role in focal adhesion formation in the human embryo fibroblasts. IP₃ stimulates release of calcium from intracellular stores and the activity of several actin binding proteins *in vitro* has been shown to be regulated by calcium concentration⁸⁴. PIP₂ itself binds to a number of actin-binding proteins *in vitro* and inhibits their interaction with actin⁸⁵ leading to the proposal that alterations in PIP₂ levels could modulate actin organization *in vitro*⁸⁶. A linkage between actin-binding proteins and PIP₂ pathway has also been sug-

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gested by Ben-Ze'ev¹. Extracellular factors alter adenylylate cyclase activity leading to change in the level of cAMP and modulating activity of protein kinase A (PKA). Activators of PKA stimulate the dissolution of stress fibres in fibroblasts, suggesting another signalling pathway in actin organization⁸⁷.

Intermediate filament proteins

In the foregoing discussion, the actin cytoskeleton is viewed extensively as a signal transducer, the organization/disorganization of which leads to changes in cell morphology and altered gene expression. It is also well known that the three cytoskeletal elements the MFs, IFs and MTs are interconnected to form a cytoplasmic network. The intermediate filament-associated protein (IFAP), plectin, has been shown to localize to focal adhesions where it seems to function as a cross-linker between IFs and actin⁸⁸.

Various experiments demonstrate that the organization of IFs is affected by hormones and growth factors, resulting in altered cell morphology and gene expression. Keratin IFs generally expressed in cells of epithelial origin, represent the most complex group of proteins in IF family. *In vivo* studies done in our laboratory suggest that the aggregation and dispersion of keratin filaments during the differentiation of rat VEC in the presence of estradiol is a consequence of successive cycles of phosphorylation and dephosphorylation of keratin polypeptides³. Modulation in intracellular levels of calcium depends on estradiol levels^{89,90} and the association of calcium-dependent cross-linking enzyme transglutaminase (TGase) with the IFs⁹¹. Keratins become relatively rigid due to phosphorylation which may facilitate cross-linking of the assembled keratins by disulphide linkages and $\Sigma(\tau\text{-glutamyl})$ lysine bonds leading to terminal differentiation of keratinocytes which assumes flattened morphology^{90,92}. Several other groups^{93,94} have demonstrated the involvement of filaggrin in the aggregation of phosphorylated keratins into filament bundles in epidermal cells.

Retinoids and progesterone diminish features of terminal differentiation and convert the cell to the secretory type. Removal of retinoids prepares the cells for terminal differentiation by inducing keratin expression at transcriptional level⁹⁵⁻⁹⁷. Many growth factors also act as modulators of IF network. EGF, transforming growth factor (TGF), keratinocyte growth factor (KGF) and cytokines can also induce alterations in cytoarchitecture. NGF is a known inducer of neurofilaments (NFs) and peripherin expression in developing neurons. Phosphorylation of NFs plays an important role in development, cross-linking and stability of the filament network with axons, this in turn regulates interactions of IFs with each other or with other cell components^{98,99}. Vimentin also undergoes alternate phosphorylation and

dephosphorylation with a looser network resulting from phosphorylation. In MCF-7 cells, a looser network of vimentin type IF is seen on estradiol treatment. It has been shown that norepinephrine induces conversion of flat sertoli cells into stellate morphology resulting from phosphorylation of vimentin¹⁰⁰. Ben-Ze'ev⁴¹ followed the regulation of synthesis of cytokeratin and desmoplakin, the proteins involved in the construction of desmosomal junctions. Along with the disruption of the desmosomes and reorganization of cytokeratins there was a dramatic decrease in the synthesis of cytokeratins and desmoplakin in the TPA-treated MDBK cells. However, Gupta *et al.*⁴ found that during keratinization of VEC, cellular connections were many fold higher in estradiol primed immature rats with increased keratin synthesis in the intermediate layers. The regulation of vimentin type IF which is coexpressed in MDBK cells was different from that of keratins and this may be due to spreading of cell on the substrate.

Microtubule proteins

Rosette and Karin¹⁰¹ have provided evidence to show that MT depolymerizing agents activate sequence-specific transcription factors such as NF-kappa B (NF-KB) and induce NF-KB dependent gene expression in HeLa cells. In the unstimulated HeLa cells they found that majority of the NF-KB resided in the cytoplasm as a complex with its inhibitor I kappa B (IKB). Upon stimulation with nocodazole the NF-KB translocates to the nucleus with a concomitant degradation of IKB. It is known that NF-KB activity is induced in presence of TPA IL-1 and certain growth factors¹⁰² and all these agents are also known to reorganize the cytoskeleton¹⁰³. Hence there is a possibility that selective depolymerization of MTs by any of these agents could be an intermediate in the signalling pathway, leading to activation of NF-KB in turn modulating gene expression to give a differentiated phenotype.

Possible interaction between cytoskeletal proteins and genes

Recently a few authors have described the probable mechanism of mammalian gene regulation by the cytoskeletal elements^{20,104}. Gene regulation in mammalian cells seems to operate at two levels. The first step is the activation of tissue-specific genes in their conversion from the sequestered to the exposed state. This involves the transfer of the appropriate DNA from the interior of the nucleus to the region of the nuclear periphery and the necessary conformational changes and specific protein interactions that render such DNA susceptible to hydrolysis by DNase I, this in turn make genes also vulnerable to inducers, repressors and other transcriptional

factors. The second step regulates the transition of the exposed genes between active and inactive states as a result of interaction with appropriate effector molecules in the surrounding medium. About 30% of the genes in mammalian cells is converted from sequestered to exposed state in a single action by cAMP, retinoic acid and NGF on transformed cells. Puck and Krystosek using Chinese hamster ovary cells²⁰ have shown that cytoskeleton network is a necessary structure in producing the specific genome exposure pattern for each cell type. It should also be noted that genome exposure is necessary but not sufficient condition for gene activation.

The mammalian cell cytoskeleton thus becomes part of an information transmission system extending from the cell membrane to its specific receptor sites through the cytoplasm and terminating in specific points on each chromosome so that specific domains of exposure and sequestration result.

Conclusion

The structural and functional integrity of a cell is maintained by the cytoskeletal network, cytoskeletal-associated proteins and the extracellular matrix proteins. Fibronectin, laminin, collagen which are regulated by hormones are connected to the actin cytoskeleton via their transmembrane receptors. The actin network is in turn linked to IFs and MTs. A coordination between these various components is necessary to maintain a definite cell shape in a given physiological condition. For instance, disruption of cytoskeletal-ECM linkage in skeletal muscle leads to sarcolemmal instability, muscle cell necrosis resulting in muscular dystrophy¹⁰⁵. In experiments with xenopus embryos, introduction of mutant E-cadherins into embryos resulted in ectodermal lesions at the beginning of gastrulation, later causing disruptions in early epidermal development¹⁰⁶. Expression of mutant N-cadherin (N-neural) lacking the ectodomain resulted in general inhibition of adhesive interaction in the embryo¹⁰⁷. Similarly disruption of cytokeratin expression during development of xenopus embryo resulted in defective gastrulation. Blessing *et al.*^{108,109} have shown that correct IF can be of critical importance for the function and stability of a given cell type.

Hormones affect their target cells during cell growth, division, differentiation, transformation and apoptosis. The mechanism involved in the cell shape change during these processes is still not clear, however, hormones are known to regulate ECM proteins which effect cytoskeletal membrane interaction bringing about altered cell morphology. The cytoskeletal associated proteins help in the polymerization/depolymerization of the cytoskeleton elements. The cytoskeleton network is an active site for signal transduction pathways. The various molecules involved in these pathways are coming to light and a

clear-cut pathway involved in hormone-induced cell shape changes is yet to be identified. However, the role of cytoskeleton, their associated proteins and ECM is well established, but how these different components coordinate to maintain a definite cell shape in a given environmental milieu is still an active field of research.

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MEETINGS/SYMPOSIA/SEMINARS

Third National Seminar on Malaria and Other Tropical Diseases

Date: 18–20 February 1997
Place: Bangalore

Topics include: Genetics and cytogenetics of insect vectors, Physiology and biochemistry; Radiation and autoradiography, Ecology and ethology, Insecticide resistance; Speciation and evolution, Chemotherapy and Immunology, Vector control – genetic, biological and chemical. Social and economic research; other related studies (insect pests of plants and animals). Epidemiology of tropical diseases; The influence of genetic and other factors on vector susceptibility to parasites; Genetic and other factors on vector susceptibility to parasites; Genetic engineering technology in vector control.

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Silver Jubilee Symposium of Catalysis Society of India: Thirteenth National Symposium on Catalysis

Date: 2–4 April 1997
Place: Dehradun

This symposium plans to cover all branches of homogeneous and heterogeneous catalysis. Particular emphasis will be given to novel applications in industrial catalysis related to petroleum refining, petrochemicals and fine chemicals. Other areas of interest are

catalysis in the protection of environment, C₁ chemistry, microporous materials synthesis and characterization.

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Short Course on Aseismic Design of Structures—Random Vibration Approach

Date: 20–22 May 1997
Place: Bangalore

Course is designed to provide a fundamental understanding of the seismic hazard analysis, theory of random vibrations, and their applications to seismic design of buildings and seismic qualification of secondary structures. It will be useful to: practising engineers or research professionals responsible for ensuring the seismic safety of a structural system or sub-system, other practising engineers requiring the application of random vibration principles (e.g. in the design of vehicular, aerospace and automobile systems), and teachers from engineering colleges.

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Implications of increasing greenhouse gases and aerosols on the diurnal temperature cycle of the Indian subcontinent

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The response to transient increase of greenhouse gases and sulphate aerosols in the Earth's atmosphere on the diurnal temperature cycle over the Indian subcontinent is examined using the data generated in coupled atmosphere-ocean model experiments. The spatial distribution of changes in seasonal mean maximum and minimum temperatures over the Indian subcontinent as simulated in combined greenhouse gas and sulphate aerosol forcing experiment for the decade 1980-89 with respect to the control climate of decade 1880-89 is found to be in fair agreement with observations. An increase in annual mean maximum and minimum surface air temperatures of 0.7 and 1.0°C respectively is projected over the land regions of the Indian subcontinent in the decade 2040s with respect to 1980s. The projected rise in maximum temperature is found to be most pronounced during the monsoon season while the rise in minimum temperature has its peak during the post-monsoon months over the region. A significant decrease in the diurnal temperature range over the Indian subcontinent during winter and no appreciable change during the monsoon season is suggested for the future.

ONE important aspect of the observed temperature change relates to its asymmetry during the day and night. Observed warming in surface air temperatures over several regions of the globe have been reported to be associated with increase in minimum temperatures (accompanied by increasing cloudiness) and decrease in diurnal temperature range¹⁻³. General circulation model simulations with increasing concentrations of CO₂ in the atmosphere have also shown pronounced increase in minimum temperatures and decrease in diurnal temperature range^{4,5}. The Indian region provides an interesting case in that while the observed mean surface air temperatures over India have been reported to exhibit a warming trend close to the global and hemispheric trends⁶, an increase in diurnal

temperature range accompanied with increasing maximum temperatures and relatively steady minimum temperatures have been reported over many parts of India⁷. In a study, Hansen *et al.*⁸ reported that the observed changes of the diurnal cycle result from a combination of local negative forcing due to sulphate aerosols over continental regions and globally-distributed positive forcing due to greenhouse gases. Model simulations of diurnal temperature range are also sensitive to parameterization of physical processes as changes in diurnal temperature range could result from changes in atmospheric water vapour, cloud optical properties, cloud cover and land surface properties. We examine here the influence of sulphate aerosols on the simulation of maximum and minimum temperatures over the Indian subcontinent using the data from a series of recent coupled climate model experiments (performed at Deutsches Klimarechenzentrum, Germany with greenhouse gas forcings as well as combined greenhouse gas and sulphate aerosol forcings). Lal *et al.*⁹ have demonstrated that the model experiment with combined greenhouse gas and sulphate aerosol forcings replicates the observed changes in surface air temperatures over the Indian subcontinent during the past century.

We discuss here the salient findings of our analysis on a comparison of area-averaged (land regions only) observed and model-simulated trends in maximum and minimum surface air temperatures and diurnal temperature range over the Indian subcontinent for the period from 1880s till 1980s. A comparison of regional trends in maximum and minimum temperatures as simulated by the model and those observed over the past century provides a potentially stringent test of the model's credibility. A comparison of the spatial distribution of observed and model-simulated changes in maximum and minimum temperatures over the region is made. A plausible future scenario of changes in maximum and minimum temperatures and diurnal temperature range for the Indian subcontinent is also presented here.

The model experiments and region of interest

The present study is based on analysis of data generated in numerical experiments performed with the European Community *HAM*burg. (ECHAM version 3) atmospheric model coupled to a Large Scale Geostrophic ocean model (hereafter referred to as ECHAM3+LSG model) at T-21 resolution. ECHAM3 is the third generation atmospheric general circulation model used for global climate modelling investigations in Germany. The LSG ocean model is based on a numerical formulation of the primitive equations appropriate for large-scale geostrophic motion. The ECHAM3 and LSG are coupled by the air-sea fluxes of momentum, sensible and latent heat, short and long wave radiation and fresh water¹⁰. For further details on the model, the reader is referred to Lal *et al.*⁹, Maier-Reimer *et al.*¹¹ and DKRZ¹².

In the first experiment (hereafter referred to as C), the control reference atmosphere has been simulated with the coupled climate model over a 170 year period (1880–2049) with constant levels of atmospheric CO₂ concentration and no anthropogenic sulphur emissions. The second experiment (hereafter referred to as G) included the observed changes in atmospheric equivalent CO₂ (CO₂ and other greenhouse gases) concentrations for the period 1880–1989 and the projected increase (based on *Business-as-usual* scenario of IPCC¹³ and representing a ~1.3% per year compound increase of CO₂) in equivalent CO₂ concentrations for the period 1990–2049. In the third experiment (hereafter referred to as S), the effects of both the greenhouse gases (equivalent CO₂) as well as the sulphate aerosols were considered. The anthropogenic sulphate burden information was obtained from Langner and Rodhe's calculations¹⁴ and the direct radiative forcing was mimicked by a change of the surface albedo following the algorithm developed by Charlson *et al.*¹⁵ The global mean sulphate forcing increased continuously since 1880s, and more rapidly from 1950 onwards. The spatial distribution of sulphate loading for the present-day atmosphere and that expected by the middle of next century over the Indian subcontinent are given in Lal *et al.*⁹

The geographic region of interest for our data analysis reported herein was mainly confined to the region bounded by ~5°N to 30°N latitude and ~65°E to 95°E longitude (Indian subcontinent and adjoining seas). It may be noted that, in spite of the fact that the horizontal resolution in the ECHAM3+LSG model experiments stated above is rather coarse (5.625° latitude, × 5.625° longitude), the model has demonstrated⁹ reasonable skill in simulating the monsoon circulation and the seasonal cycle of observed climatology for the region.

Comparison of past observed and simulated regional diurnal temperature cycle

Observed and model-simulated trends in maximum and minimum temperatures

Analysis of the recent simulated global and Northern Hemispheric mean near-surface temperatures has proved that an anthropogenic climate change signal in observed records of near-surface air temperature change can be detected at 95% confidence level¹⁶. The study also suggests that model-simulated past trends in global mean surface warming can be reconciled with the observed warming trends only when the cooling effect of aerosols on climate is accounted for. In order to place a high degree of confidence in future climate change, climate models must replicate the past trends in key climate elements within the limits of natural variability. In this respect, the year-to-year variability in model-simulated surface air temperature and monsoon rainfall over the Indian subcontinent has been found to be in fair agreement with the observed climatology⁹.

Figure 1 depicts the 5-year running mean anomalies in area-averaged maximum and minimum surface air temperatures as observed⁷ for the period from 1901 to 1994 and as simulated by the model for the period 1880–2049 in experiments G and S for the Indian subcontinent (land points only). The observed series⁷ is based on spatial means of 121 stations over India while the model simulated data series is based on the spatial average of 19 land points only. On regional scales, the higher level of year to year variability obscures any systematic differences in maximum and minimum surface air temperatures simulated in experiments G and S in the first half of this century. All India mean annual maximum temperature, has a significant trend of 0.6°C/100yr in observational records while the minimum temperature is found practically trendless⁷. Both the annual mean maximum and minimum temperature anomalies in experiment G show a much higher increasing trend (0.85°C/100yr in maximum temperature and 0.84°C/100yr in minimum temperature respectively) than observed in the past century. In experiment S, the annual mean maximum temperature anomalies exhibit an increasing trend of 0.61°C during the past century which is in excellent agreement with observed trends. The annual mean minimum temperature anomalies have an increasing trend of 0.18°C during the past century in experiment S. Moreover, both the maximum and minimum temperature anomalies simulated by the model in experiment S are found to be closer to the observed anomalies in the recent years, thus signifying the importance of aerosol forcing (Figure 1). The inter-annual variability generated by the model is found to be rather large in a statistical sense as compared to that

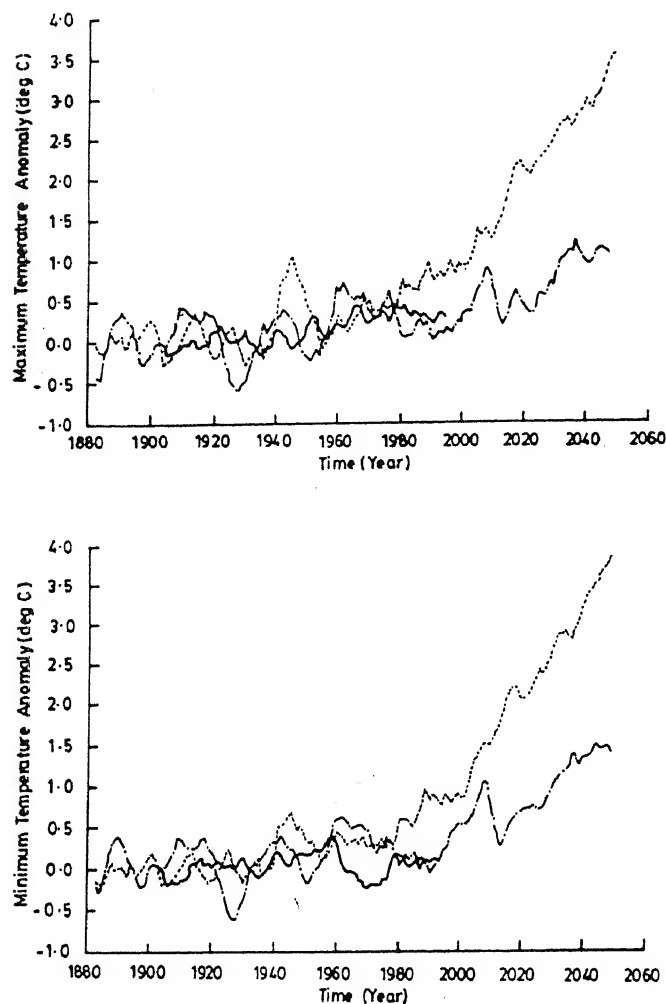


Figure 1. Observed (1901–1994) and model-simulated (1880–2049) trends in area-averaged anomalies in annual mean maximum (upper) and minimum (lower) surface temperatures (5 year running means, land regions only; °C) over the Indian subcontinent. (Solid thick line is for observed anomalies; solid thin line is for anomalies simulated in CO_2 + aerosol experiment and dotted line is for CO_2 only experiment.)

observed over the region even though we recognize that these climate model simulations do not attempt to precisely simulate individual years.

Table 1 gives the mean annual and seasonal (winter and monsoon) changes in area-averaged maximum and minimum surface air temperatures in the decade 1980–89 with respect to the decade 1880–89 over the Indian subcontinent as simulated in the experiments G and S and the observed⁷ linear trends per 100 year period. While the diurnal temperature range over the region simulated by the model in experiment G is found to decrease (during winter) or remain unchanged (during monsoon) in the past century, it has increased on seasonal as well as on annual mean basis in experiment S which is in agreement with observations (but unlike the observed and/or simulated trends over many parts of the globe).

Two important points emerge from Table 1. The first is that the minimum temperature changes simulated by the model in a season or on annual mean basis in the greenhouse gas forcing only experiment are significantly higher when compared to the observed changes over the past century over the Indian subcontinent. The increasing trends in maximum and minimum temperatures simulated in experiment S are lesser as compared to G due to negative forcing exerted by the introduction of sulphate aerosols. The combined greenhouse gas and sulphate aerosol forcing experiment is able to replicate the observed changes in maximum and minimum temperatures over the region in a broad sense on seasonal as well as on annual mean basis. The second point is that, while the differences in maximum temperatures simulated in experiments S and G are less pronounced, a relatively smaller increase in minimum temperature is simulated in experiment S as compared to G on seasonal as well as annual mean basis. This could have been induced by a reduction in high cloud amount as, in high clouds, an increase in albedo could be offset by a change in cloud infrared emissivity, leading to decrease in warming effect during the day and increase in cooling effect during the night¹⁷. This further suggests the possibility that the direct influence of sulphate aerosols (trapping of solar radiation) may not be as dominating as the indirect effect (through its influence on clouds). The general notion that an increase in cloud cover takes place due to industrial sulphur emissions³ may not be valid over the monsoon region. The significant decline in the nighttime temperatures in experiment S is supported by a reduction of about 0.8% in area-averaged fractional cloud cover in the decade 1980s with respect to the control climate of 1880s over the monsoon region. Decreased cloud cover and consequent suppressed water vapour feedback in a relatively drier atmosphere should produce lower minimum temperatures. The precise modulating role of clouds in numerical models is, however, not yet fully understood. The results from experiment S are in conformity with the observations in that both exhibit an increase in diurnal temperature range accompanied with increasing maximum temperatures over the region.

Spatial distribution of observed and model-simulated changes in diurnal temperature cycle

A comparison of spatial patterns of observed and model-simulated (Experiment S) maximum and minimum temperatures and diurnal temperature range over the region of interest for the present-day atmosphere (1980s) suggests reasonable similarity between them during both winter and monsoon seasons (Figures 2 and 3). During winter, the simulated maximum temperatures are lower and simulated minimum tem-

Table 1. A comparison of model-simulated and observed linear trends ($^{\circ}\text{C}$) per 100 years in area-averaged (land only) maximum and minimum surface temperatures over the Indian subcontinent (based on data from 1901 to 1990)

Period	Experiment G		Experiment S		Observed	
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
Annual	0.85	0.84	0.61	0.18	0.6	0.1
Winter (DJF)	0.86	0.97	0.78	0.29	0.9	0.2
Monsoon (JJAS)	0.83	0.83	0.37	0.08	0.4	-0.3

temperatures are higher than the observed climatology (Figure 2). Consequently, the simulated diurnal range is lower than the observed. A better agreement is found between the spatial patterns of observed and simulated magnitude of maximum and minimum temperatures during the monsoon season (Figure 3). The model due to its rather coarse resolution has not been able to realistically portray the observed locations of extremes in maximum or minimum temperatures. The scanty observations limit verification of the model simulations of the diurnal temperature cycle in the Himalayan region.

In order to quantify the similarity between the observed and model-simulated spatial distribution of maximum and minimum temperatures, we performed calculations of the pattern correlation coefficient and root mean square error between the observed and model-simulated climatology of maximum and minimum temperatures for winter and monsoon season over the region of interest. For this purpose, both the observed and model-simulated data were first interpolated on to a common 2.5° square grid configuration with a cubic spline fit. The pattern correlation coefficient gives a measure of similarity of the pattern structure of the observed and simulated fields throughout the region whereas the root mean square error gives an overall measure of the absolute error in simulating the field over the region. During winter, the pattern correlation coefficient between the observed and simulated climatology is 0.61 for maximum temperature and 0.69 for minimum temperature. The root mean square error is 3.6°C for maximum temperature and 2.9°C for minimum temperature. During monsoon season, the correlation coefficients are 0.63 and 0.59 while the root mean square errors are 3.7°C and 4.1°C for maximum and minimum temperatures respectively. Keeping in view the fact that the spatial distribution of model-simulated temperatures

considered here are based on only 19 land points as against the spatial distribution of observed climatology

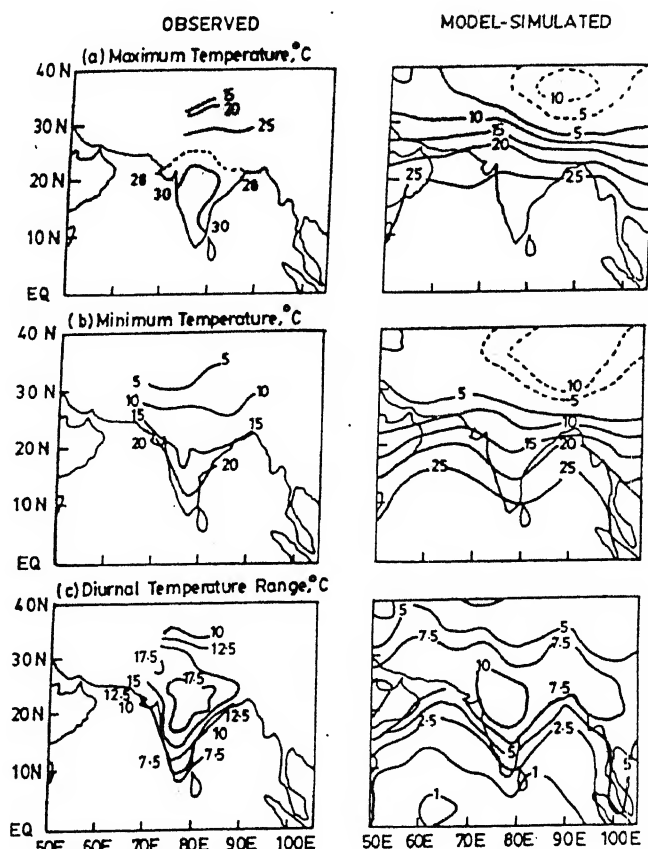


Figure 2. Spatial distribution of present-day (1980s) observed and model-simulated (under combined equivalent CO_2 and aerosol forcings) maximum and minimum temperatures and diurnal temperature range ($^{\circ}\text{C}$) over the Indian subcontinent during winter season.

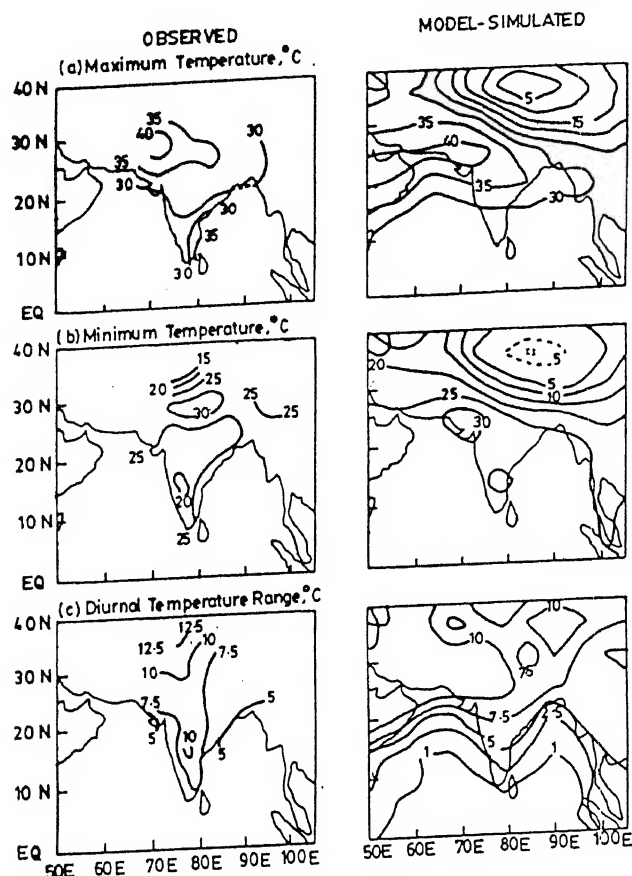


Figure 3. Spatial distribution of present-day (1980s) observed and model-simulated (under combined equivalent CO_2 and aerosol forcings) maximum and minimum temperatures and diurnal temperature range ($^{\circ}\text{C}$) over the Indian subcontinent during monsoon season.

ogy based on data from 121 stations, the pattern correlations higher than 0.6 suggest that model simulations perform reasonably well in simulating the maximum and minimum temperature patterns over the region.

The spatial distribution of observed⁶ changes in the annual mean temperatures in the decade 1980s with respect to 1880s over the Indian subcontinent suggested a rising trend in the maximum temperature at almost all stations south of 23°N and a decreasing trend north of 23°N . The minimum temperatures showed increasing trend in almost all parts of central and Peninsular India barring some pockets along west and east coasts with nominal falling trend. Decreasing trends in the simulated maximum and minimum temperatures over the north India and increasing trends over the Peninsular India in the experiment S are in fair agreement with observations (Figure 4) except in the Himalayan highlands. The differences in the highlands could be due to sparse observations and also due to coarse resolution of the model over the region. The experiment with only CO_2 forcing produced consistently higher than realized changes in maximum and minimum temperatures over

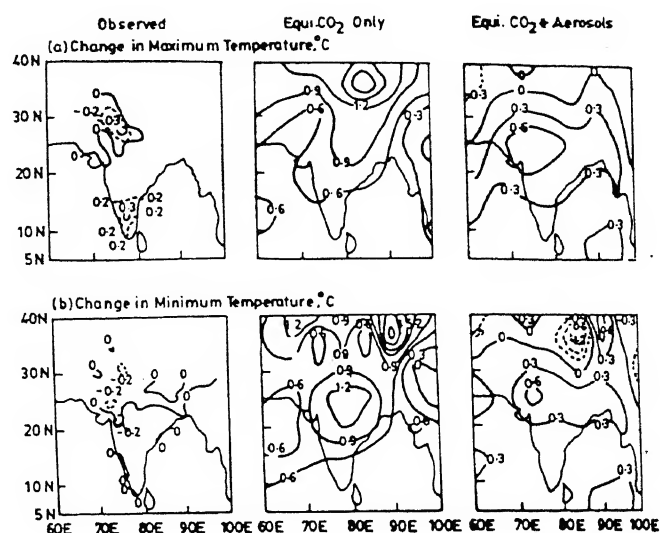


Figure 4. Spatial distribution of observed and model-simulated (equivalent CO_2 forcing and combined equivalent CO_2 and aerosol forcings) changes in annual mean maximum (a) and minimum (b) surface temperatures ($^{\circ}\text{C}$) in the decade 1980s with respect to 1880s over the Indian subcontinent.

the region on annual mean basis. The combined CO_2 and aerosol forcing is able to reproduce the observed changes in diurnal temperature cycle during the past century better than the CO_2 forcing only (Figure 4). Our findings on the future projections of regional changes in maximum and minimum temperatures and diurnal temperature range based on the combined CO_2 and aerosol forcing experiment should, therefore, have a better degree of confidence.

Future changes in diurnal temperature cycle

Table 2 gives the area-averaged annual and seasonal changes during the decade of 2040s over the Indian subcontinent in the maximum and minimum temperatures as simulated by CO_2 forcing only as well as by combined CO_2 and aerosol forcing. The projected annual mean changes in maximum and minimum temperatures in CO_2 forcing only experiment are more than a factor of two higher relative to those in combined CO_2 and aerosol forcing experiment.

In the combined CO_2 and aerosol forcing experiment, while the projected rise in maximum temperature is found to be most pronounced during the pre-monsoon and monsoon seasons, in minimum temperature it is for the post-monsoon and winter months. A decline in the diurnal temperature range is simulated by the model for the decade 2040s on annual mean basis. The projected decline in diurnal temperature range during winter is most pronounced (0.6°C) and

Table 2. Changes ($^{\circ}\text{C}$) in model-simulated area-averaged maximum and minimum temperatures (land only) during 2040s as compared to 1980s over the Indian subcontinent

Period	Equivalent CO_2		Equivalent CO_2 + aerosol	
	Maximum	Minimum	Maximum	Minimum
Annual	2.38	2.73	0.74	1.01
Winter (DJF)	2.60	2.62	0.47	1.10
Pre-monsoon (MAM)	3.23	3.15	0.87	0.99
Monsoon (JJAS)	1.61	2.50	0.88	0.88
Post-monsoon (ON)	2.32	2.96	0.64	1.17

is found to be statistically significant at 90% confidence level. During the monsoon season, no change is simulated in the diurnal temperature range for the future.

In its spatial distribution, while no significant rise in maximum temperature is expected over northeast India by the middle of next century, a rise of about 1.5°C is simulated along the western margins of India (Figure 5). A marginal decline in the minimum temperature of about 0.5°C is likely over the northeast India whereas a rise of above 1.5°C is possible along the western Indian subcontinent. The model-simulated changes in both maximum and minimum temperatures are found to be characteristically smaller over northeast India compared to rest of the region. The model results suggest a decline in diurnal temperature range ($\sim 0.5^{\circ}\text{C}$) over most parts of India due to relatively larger increase in the minimum temperature. The diurnal temperature range over northeast India may, however, increase by about 0.5°C .

Discussions and conclusion

A comparison of observed and model-simulated trends in annual mean area-averaged maximum and minimum surface temperatures suggests that the combined greenhouse gas and sulphate aerosol forcing experiment has been able to reproduce the observed changes in diurnal temperature cycle over the region during the past decades in a broad sense. The spatial distribution of changes in annual and seasonal mean, maximum and minimum temperatures over the Indian subcontinent as simulated in combined greenhouse gas and sulphate aerosol forcing experiment for the decade 1980s with respect to the control climate of decade 1880s is found to be in fair agreement with observations. Thus, the inclusion of aerosol forcings in coupled climate model experiments has

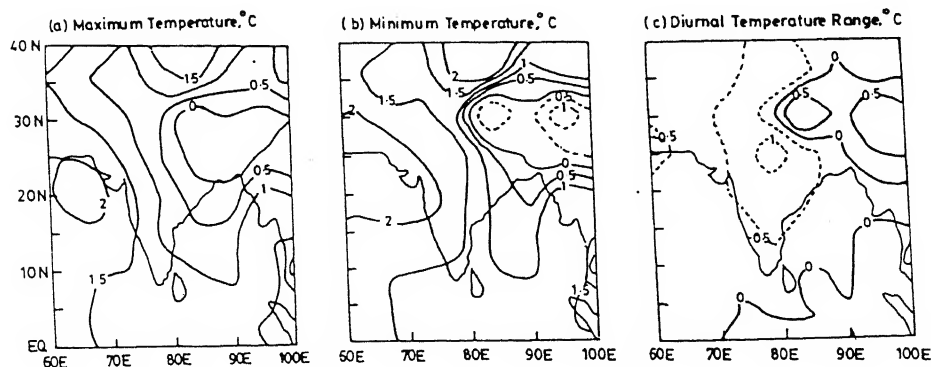


Figure 5. Spatial distribution of changes in annual mean maximum and minimum temperatures and diurnal temperature range ($^{\circ}\text{C}$) during the decade 2040s with respect to the decade 1980s over the Indian subcontinent as simulated by the model due to combined equivalent CO_2 and aerosol forcings.

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provided us some insights on how to explain, in general, the observed asymmetry in the diurnal temperature cycle over the region.

An increase in annual mean maximum and minimum surface air temperatures of 0.7 and 1.0°C respectively over the land regions of the Indian subcontinent by the middle of next century is simulated by the model taking into account the projected emission of greenhouse gases and sulphate aerosols. The projected rise in maximum temperature is found to be most pronounced during the monsoon season while the rise in minimum temperature has its peak during the post-monsoon months over the region. A significant decrease in the diurnal temperature range over the Indian subcontinent during the winter season and no appreciable change during the monsoon season is simulated. The projected changes in the maximum and minimum temperatures and the diurnal temperature range could have significant impact on the patterns of agricultural productivity over the Indian subcontinent.

The key factors that affect the regional scale performance of global climate models are the horizontal resolution and physical parameterization schemes. The coarse resolution may introduce systematic errors in the depiction of coastlines as well as high mountains with consequent effects on the simulation of regional circulation and temperatures. In this respect, the projections given here have only a limited degree of confidence. Since, at present, only the direct effects of sulphate aerosols are considered in model experiments (in terms of surface albedo), we cannot judge precisely the influence of aerosols in modulating the cloud microphysics. When the resolution of available climate models increases with the availability of enhanced super-computing power and improved parameterization schemes of subgrid-scale physical processes such as clouds are incorporated, a general increase in accuracy in regional projections may be expected.

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Forcing mechanisms of the Bay of Bengal circulation

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A state-of-the-art ocean general circulation model, set up for the North Indian Ocean and driven by climatological wind stress simulates most of the observed features of the near-surface circulation of the Bay of Bengal. The prominent features of the annual cycle are an anticyclonic gyre with a poleward East India Coastal Current (EICC) during February–May, and an equatorward EICC during October–December. During the summer monsoon, the coastal current flows poleward in the south and equatorward in the north. To identify the principal mechanisms governing this cycle, we carried out experiments with modified winds. When spatially uniform wind stress was applied only over the Bay, the circulation is similar to, but weaker than the observed, and can be linked to two coastal Kelvin wave pulses which originate along the eastern boundary of the Bay during the summer and winter monsoons. When the Bay is forced with observed winds, the wind stress curl strengthens the poleward EICC during February–May and the equatorward EICC during October–December. The principal contribution of equatorial winds is to generate the equatorward coastal current during the summer monsoon off the east coast of India.

1. Introduction

THE Indian Ocean has a unique geographic setting; it is bounded in the north by the Eurasian Land mass. This termination of the ocean in the tropics gives rise to spectacular seasonality in the overlying atmosphere and, consequently, in the ocean itself. North of 10°S the surface winds blow from the southwest during May–September and from the northeast during November–February. The ocean is primarily driven by winds, and under the influence of the seasonally reversing monsoon winds, the circulation in the Indian Ocean reverses twice during the year.

The response of the Indian Ocean to monsoon forcing has evoked considerable interest in the past. However, most of the attention has been focused on the western part of the Indian Ocean, which has a prominent seasonally reversing current viz. the Somali Current^{1,2}. The rest of the basin has remained rather poorly observed

and little studied. This is particularly true for the Bay of Bengal, which forms the northeastern part of the Indian Ocean. The Bay is not only of great oceanographic interest but it also plays an important role in maintaining and modulating the monsoon over the Indian region.

The Bay is markedly different from the Arabian Sea in two respects. Firstly, over the Arabian Sea, evaporation exceeds precipitation and it receives highly saline water from the Red Sea and from the Persian Gulf, making the salinity of the upper layer rather high. In contrast, over the Bay rainfall exceeds evaporation and the Bay also receives a large quantity of freshwater as river discharge, leading to an upper layer of less saline water for a large part of the year³. The surface waters in the Bay have such low salinity that the stratification in the upper layer is dominated by salinity gradients rather than temperature gradients⁴.

Secondly, over a large part of the year the sea surface temperature (SST) of the Bay is above 28°C (ref. 5), which is the threshold SST for active generation of large-scale convection/precipitation⁶. On the other hand, the SST in the Arabian Sea, west of about 70°E, is below the threshold over most of the year. The SST is determined by the heat flux at the sea surface (which in turn depends on surface winds and on SST itself) and horizontal and vertical advection in the ocean. How SSTs higher than 28°C are maintained in the Bay is not yet clearly understood. Most of the low pressure systems, depressions and cyclones which give rainfall over the Indian region are generated over the Bay. The coupling of the monsoon atmosphere with the Bay must therefore play an important role in determining rainfall over the Indian region and its variability. Thus understanding the Bay is a challenging problem in the areas of ocean dynamics and coupled ocean–atmosphere systems.

During the last few years, new observations have been made in the Bay, by Shetye *et al.*^{4,7,8} in the western Bay and by Murthy *et al.*⁹ and Suryanarayana *et al.*¹⁰ in the central Bay. These and earlier analysis of ship-drifts¹¹ show a distinct seasonal cycle of surface circulation³. A prominent feature of this seasonal cycle is the seasonally reversing coastal currents. The coastal current along the

east coast of India flows poleward before the summer monsoon and equatorward during the winter monsoon season. During the summer monsoon there is a poleward current along the Indian coast in the southern part and an equatorward current in the northern part.

One of the major aims of ocean modelling studies is to simulate the observed seasonal variation in the circulation and understand it in terms of the response to the winds over the Bay as well as other regions. Both these aspects have been addressed so far using the relatively simple reduced gravity models, which assume that the ocean is made up of very few layers of which the lowest one is static. The studies of Potemra *et al.*¹² and McCreary *et al.*¹³ (henceforth MKM) have shown that major features of the surface circulation are simulated by such models. Two types of waves have been shown to play a pivotal role in the time-dependent response of the Bay to the wind-forcing. These are the Rossby waves which occur everywhere in the oceans, and the Kelvin waves, which propagate along the coast. Equatorial Kelvin waves generated by winds over the equatorial Indian Ocean propagate eastward. On encountering the eastern boundary of the ocean, a part of the energy of these waves is transferred to coastal Kelvin waves. The latter move rapidly along the coastline of the Bay of Bengal and influence the circulation in the Bay.

On the question of forcing the circulation in the Bay, different mechanisms have been proposed involving winds over the Bay, and winds over the equatorial Indian Ocean, and Rossby and Kelvin waves. Potemra *et al.*¹² highlighted the role of planetary waves in setting up the seasonal circulation. Yu *et al.*¹⁴ argued that these waves have their origin as equatorial Kelvin waves, implying that equatorial forcing alone can set up the observed annual cycle. MKM¹³ highlighted the importance of coastal Kelvin waves and Rossby waves generated entirely in the Bay by local alongshore winds. Shetye *et al.*⁷ have emphasized the role of winds over the open Bay. Recently, Shankar *et al.*¹⁵ and McCreary *et al.*¹⁶ have examined the roles of different mechanisms in an analytic model and in numerical experiments.

In this paper we address the problem of understanding the relative roles of different mechanisms in driving the circulation of the Bay using a state-of-the-art ocean general circulation model (OGCM) developed by Bryan¹⁷ and Cox¹⁸. While general circulation models of the atmosphere have been used in our country for several years, this is the first implementation of an OGCM. We report the results of several experiments carried out with the Bryan-Cox model with two objectives. The first is to see how well the known features of the near-surface circulation in the Bay are reproduced when climatological monthly mean winds force the model. Second, we carry out numerical experiments aimed at constructing a simple, yet dynamically correct, picture of circulation in the Bay. These experiments also permit us to estimate the

contribution of different mechanisms in driving the circulation in the Bay. The model set-up is briefly described in section 2. Section 3 discusses the annual cycle, comparing the model results with ship-drifts. Sensitivity of the model to horizontal resolution is examined in section 4. In section 5 the results of model runs with modified wind fields are examined. Section 6 summarizes the main findings.

2. The model set-up

The Bryan-Cox model, now commonly known as the Modular Ocean Model¹⁹, solves the primitive equations on a finite difference grid using the method of Bryan¹⁷. The model domain for the present study lies between 20°S–25°N and 40°E–100°E, with a no-slip condition on all boundaries. The horizontal grid spacing is 1° in both zonal and meridional directions. There are 15 levels in the vertical, of which 8 are in the top 100 m (Table 1). We have also made a run with a grid spacing of 1/3° to test the sensitivity to horizontal resolution.

Horizontal diffusivity and viscosity are set to $5 \times 10^7 \text{ cm}^2 \text{ s}^{-1}$. The scheme of Pacanowski and Philander²⁰ is used for vertical mixing. The model has realistic coastline geometry and topography as resolved by the Scripps topography²¹. The model is started from a state of rest with initial conditions for temperature and salinity obtained from the Levitus²² climatology for January. At the surface the model is forced by the climatological wind stress of Hellerman and Rosenstein²³ (henceforth H&R). The surface boundary condition for temperature and salinity is of the relaxation type²⁴, with a relaxation time of 10 days. At the southern boundary, temperature and salinity are relaxed to their climatological values. It is integrated for four years and model fields during the fourth year are used for the discussion below.

Table 1. Vertical levels in the model

Level	Thickness (m)	Depth to the grid point (m)
1	10.0	5.0
2	10.0	15.0
3	10.0	25.0
4	10.0	35.0
5	10.2	45.1
6	10.9	55.7
7	13.5	67.9
8	21.4	85.3
9	42.5	117.3
10	90.7	183.9
11	184.1	321.3
12	336.6	581.3
13	542.7	1021.3
14	765.5	1675.4
15	941.8	2529.1

The geometry of the model is unrealistic with a closed southern boundary, no Indonesian throughflow, closed Malacca strait, lack of island chains and smooth bottom topography. There is no evidence that effects arising from 20°S influence near-surface circulation in the Bay. Similarly, the closing of the Indonesian passage is unlikely to affect the Bay of Bengal circulation because the Indo-Pacific throughflow is found to affect the circulation only to the south of the equator¹³. However, consequences of closing the Malacca Strait and lack of island chains in the model are not known. A deficiency of the model is the lack of river discharge, though it is partly accounted for by the surface boundary condition on salinity. Since the present study is the first attempt to study the Bay using an OGCM we decided to defer exploring the role of river discharge to the future.

3. The annual cycle

During May to September the southeast trades in the south Indian Ocean cross the equator near the African coast and turn southwesterly. In the Bay of Bengal and Arabian Sea the southwesterlies strengthen during June, reach their peak during July–August, start decaying during September, and vanish by October (Figure 1). During November–February northeasterly winds prevail north of the equator, turning northwesterly in the south. These winds reach their peak during December. In the Bay these winds die by the end of February (Figure 1), whereas in the Arabian Sea they persist till March. In general, the winds during November–February are much weaker than the summer monsoon winds.

Winds in the equatorial (5°S–5°N) Indian Ocean (EIO), east of 55°E, are relatively calm throughout the year except during the transition between the monsoons, April–May and October–November, when strong west-lies occur²⁵. Due to these episodes, the annual cycle of the winds in the EIO has a significant semi-annual component. In contrast, the periodicity of winds in the Bay of Bengal is predominantly annual.

In response to the winds, circulation in the Bay exhibits a seasonal cycle both in surface currents and in deeper flow. The ship-drift climatology¹¹ provides a comprehensive view of the seasonal cycle of the surface currents in the Bay of Bengal. We first examine how well the model performs in simulating the upper layer circulation by comparing with ship-drifts.

Comparison with ship-drifts

During February–April ship-drifts in the Bay show (Figure 2a, b) the presence of an anticyclonic (clockwise) gyre with a poleward East India Coastal Current (EICC). To the south of the gyre, approximately along 6°N, a westward current called the North Equato-

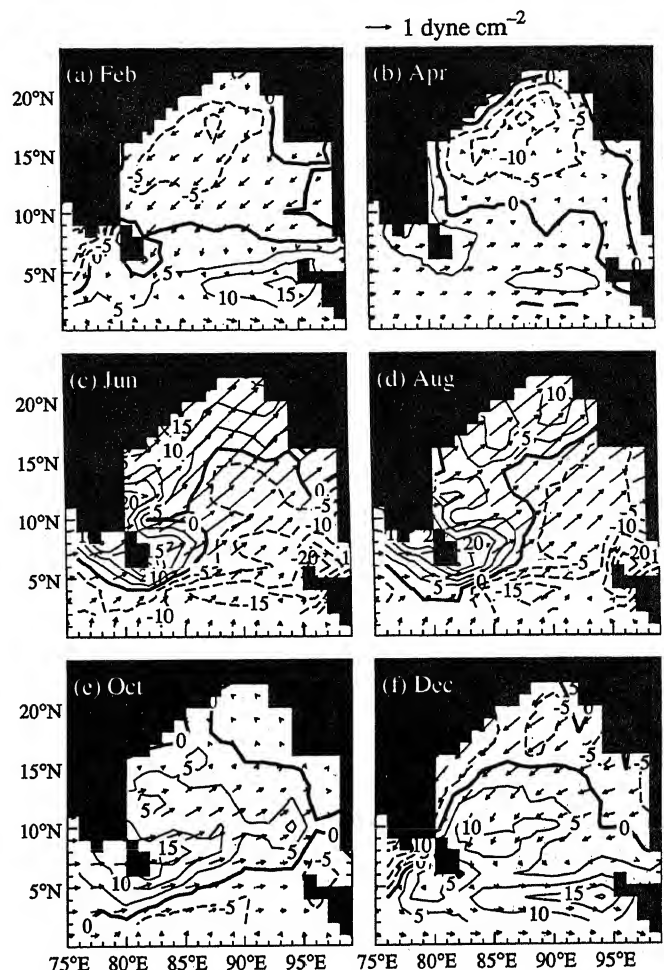


Figure 1. Bi-monthly maps of winds over the Bay of Bengal (H & R). Vectors represent wind stress. Contours represent wind stress curl; thick, dashed and continuous contours represent zero, negative and positive curl respectively. Contour interval is 5 dynes cm⁻³.

rial Current²⁶ (NEC) is present. Piecing together hydrographic data and climatologies^{11,22} Shetye *et al.*⁷ inferred that the poleward EICC in the Bay of Bengal²⁷ during February–May is a part of a seasonal anticyclonic gyre enveloping the entire Bay. The anticyclonic gyre, the EICC and the NEC are reproduced well by the model. The initial formation of the anticyclonic gyre in the model takes place in the northern Bay during January. The EICC forms by February (Figure 2a) and is fully developed by March. During February, the northern part of the EICC is fed by a northwestward flow in the central Bay and the westward North Equatorial Current feeds into the southern part. The model EICC and the anticyclonic gyre are present up to level 10 (about 200 m). Typical speeds are in the range 15–30 cm s⁻¹. During February and March there is a northwestward flow in the southwestern Bay and during April a slow southward drift exists in the rest of the open Bay

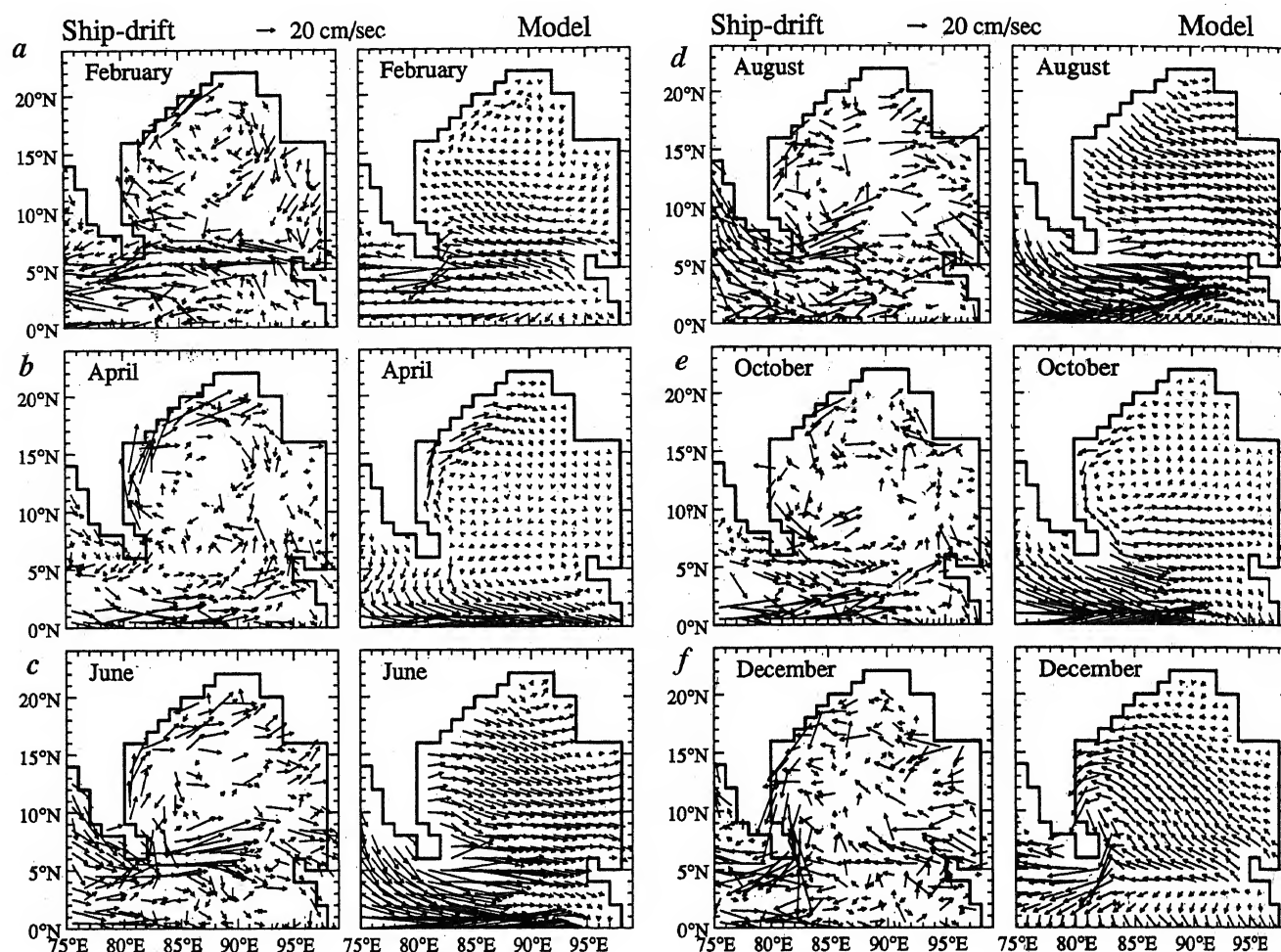


Figure 2. Comparison of the model currents at level 1 (5 m) with ship-drifts. Left panel shows ship-drifts and right panel shows model currents. Five day outputs from the model are averaged to construct monthly mean model currents. To project dominant currents a ship-drift vector was plotted only when the magnitude exceeded 5 cm s^{-1} and when there were more than 5 observations in a 1° box.

(Figure 2 b). The simulated open ocean flow is weaker than the ship-drifts. The ship-drifts suggest a northeastward flow in the southeastern Bay. This flow is very weak in the model. Further, the ship-drifts between 0 and 3°N are eastward, whereas the model flow is towards the southeast.

The ship drifts show that by May the anticyclonic gyre begins to break up and is no longer seen by June (Figure 2 c). A poleward coastal current is seen along the east coast of India during May–June but it cannot be associated with a gyre like in March–April. In the open Bay, in the north, large eastward ship drifts are observed during June–August (Figure 2 c, d). These are probably due to Ekman drift, i.e. directly wind-driven shallow currents, flowing at an angle to the right of the wind in the northern hemisphere. In the model also, an eastward flow is seen in the open Bay during this period. However, the model currents at the first three model levels (5, 15 and 25 m) appear to be dominated by Ekman

flow²⁸, and hence do not compare well with observations. The model Ekman flow is too strong and makes too large an angle with the wind direction. The model eastward current south of Sri Lanka has shifted southward of the observed location. The prominent northeastward flow into the Bay at the termination of this eastward current is not simulated at all. The current along the east coast of India is also not simulated. Clearly the model needs to be improved for a more realistic simulation of the upper layer currents in this season.

Immediately below the layer dominated by Ekman flow (at 35 m), however, the currents along the east coast of India during May and June are well simulated by the model (Figure 3 a, b). At this level there is also a poleward current along the eastern boundary of the Bay. As summer progresses, this current propagates along the boundary of the basin with the coast to its right; by July it is an equatorward flow off the Orissa

4. Sensitivity to horizontal resolution

Since the fundamental length scale of ocean motions, the Rossby radius of deformation, can be smaller than 1° in the latitude range of the Bay of Bengal, a better horizontal resolution is desirable. But the computational load rises steeply with increase in number of grid points and decrease in time step demanded by the numerical stability requirements. As a compromise we set up the model with a minimum spacing of $1/3^\circ$ in the Bay of Bengal and in the EIO. With a grid spacing of $1/3^\circ$, most energetic small-scale features of the flow, including the equatorially trapped waves (which are crucial to the forcing mechanisms described in next section) can be expected to be well represented²⁹. Horizontal eddy coefficients in this case are $2 \times 10^7 \text{ cm}^2 \text{ s}^{-1}$. To save computer time, the depth averaged flow – ‘the barotropic component’ – is suppressed. Since our interest in this study is limited to the upper layers, where the contribution from the depth averaged velocity is small, this is a reasonable strategy to adopt.

One immediate consequence of increasing spatial resolution to $1/3^\circ$ is that the flow field acquires rich spatial structure with embedded eddy type flow pattern, particularly during February–March and June–September (Figure 4). The northwestward flow in the central Bay splits into two branches (Figure 4a, b). The main branch turns northward to feed the EICC between 14°N and 17°N . A small branch forms a cyclonic eddy offshore of the EICC between 10°N and 13°N . During July two anticlockwise cells are present along the western boundary (Figure 4b); the first is located off the Sri Lankan coast and the second off the southeast coast of India. How realistic is this fine structure? We really do not know, but there are references in the literature to similar, relatively small scale features in the Bay of Bengal circulation. Legeckis²⁷ observed in satellite derived SST that eddies characteristic of western boundary currents are present in the Bay of Bengal as well. Babu *et al.*³⁰ documented a subsurface cyclonic eddy along the western boundary during summer monsoon. The flow field along the western boundary during the summer monsoon consists of several cells in the observations of Shetye *et al.*⁴. It is hoped that the present fine-resolution model results would serve as a guideline to plan future field experiments in this direction. Since the wind stress used to force the model does not have the small spatial scales seen in Figure 4a, c, it is suggested that these small-scale structures in the flow field are born from ocean dynamics. This needs to be confirmed through further study.

5. Forcing mechanisms

Modelling studies during the last few years^{12–16} have shown that the main features of the annual cycle of cir-

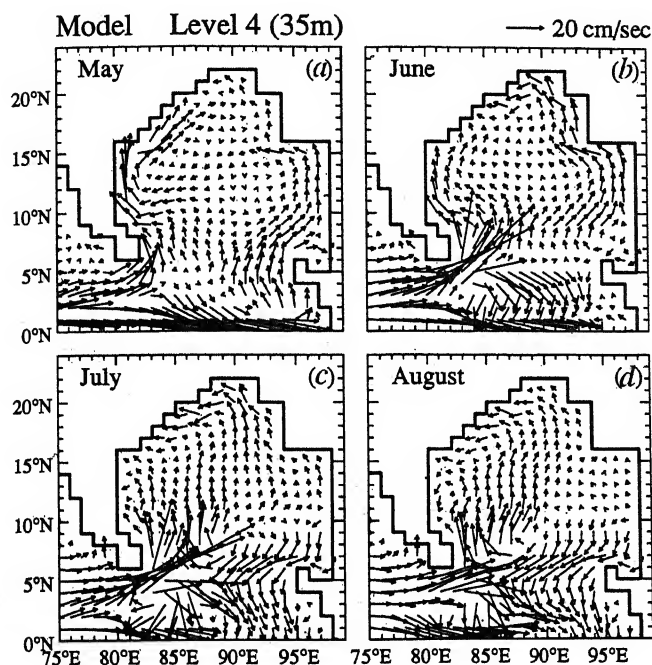


Figure 3. Model currents at level 4 (35 m) during (a) May, (b) June, (c) July, and (d) August.

coast. On the other hand, the poleward current along the east coast of India retreats and disappears by July. The flow field inferred from hydrographic observations along the western boundary of the Bay⁴ supports the model simulation. There are no observations in the eastern part of the Bay to confirm the presence of the poleward current predicted by the model. The model northward flow in the open Bay in July at this level is consistent with observations⁹.

Both model currents and ship-drifts suggest a cyclonic gyre during October–November. This gyre breaks up and a northwestward flow develops in the open Bay during November. Hydrographic data⁸ during December also suggests a similar feature. The formation of a southward coastal current off the east coast of India takes place in the model during October (Figure 2e), after the withdrawal of southwesterly winds. This southward coastal current is present till January.

In summary, we note that the overall pattern of surface currents in the model is consistent with ship-drifts. However, there are differences near the western and southern parts of the Bay during May–September, when the winds are strongest. Both the model level-1 currents and the observed surface circulation are best organized during March–April, when an anticyclonic gyre with a poleward EICC is present. There is also a period of organized cyclonic flow with an equatorward EICC, during October–November.

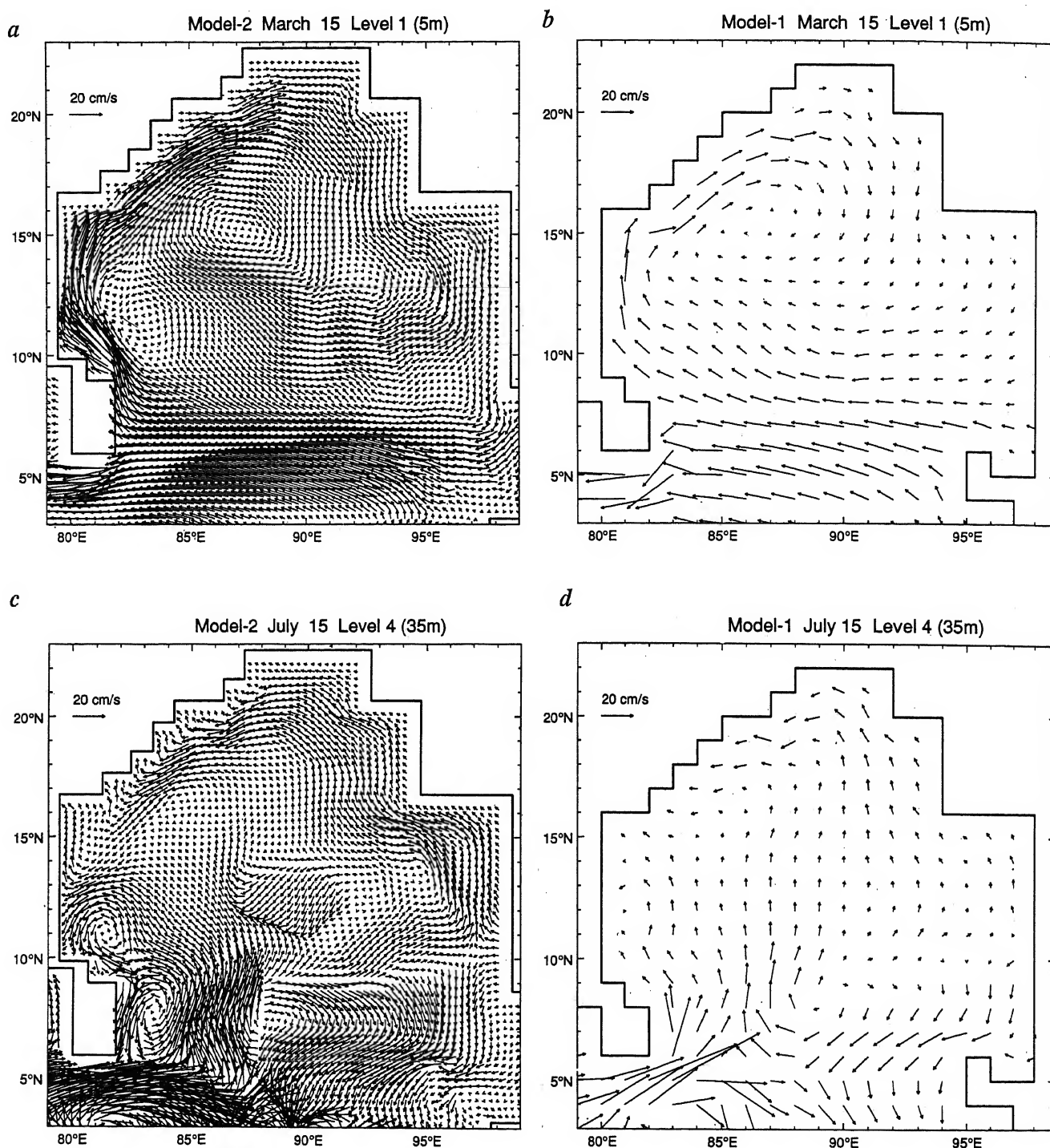


Figure 4. *a*, Currents during March at the first model level (5 m) from the model with a horizontal grid of $1/3^\circ$ in the Bay of Bengal and in the EIO; *b*, Currents during March at the first model level (5 m) from the model with a horizontal grid spacing of 1° over the entire model domain; *c*, Currents during July at the fourth model level (35 m) from the model with a horizontal grid spacing of $1/3^\circ$ in the Bay of Bengal and in the EIO; *d*, Currents during July at the fourth model level (35 m) from the model with a horizontal grid spacing of 1° over the entire model domain.

ulation in the upper Bay can be understood in terms of coastal Kelvin waves along the periphery of the Bay and westward-propagating Rossby waves. The mechanisms that have been identified as generating the Kelvin waves

are alongshore winds in the Bay¹³ and equatorial processes^{12,14}. Rossby waves are generated in the Bay either by Kelvin waves moving along the eastern boundary of the Bay or by the vorticity of the winds (wind stress

curl) over the Bay. We thus have three mechanisms – longshore winds, wind stress curl and equatorial processes – that determine the annual cycle of circulation in the Bay.

In this section we report results of numerical experiments conducted to assess the relative roles of these forcing mechanisms. The model described in section 4 was used for these experiments. In the first experiment winds everywhere except the Bay were switched off; the winds over the Bay being averaged in space to eliminate wind stress curl. The purpose of this experiment was to examine what aspects of the circulation in the Bay can be reproduced with spatially uniform, but seasonally varying winds. In the second experiment, winds were again switched off everywhere except the Bay; the wind stress over the Bay, however, was taken from the monthly mean wind stress climatology²³. In the third experiment winds everywhere were from climatology. The difference between the velocity field in the third and second experiments allows us to identify the contribution to the Bay from elsewhere in the Indian Ocean, primarily from the equatorial belt.

Circulation in the Bay due to curl-free winds over the Bay

Convergence (divergence) in the upper ocean due to wind stress arising from a wind field that possesses curl, induces Ekman pumping (suction) in the deep ocean. Wind stress in the presence of the β -effect (variation of the coriolis parameter with latitude) also gives rise to Ekman pumping (suction)³¹. The major contribution to the Ekman pumping (suction) comes from the curl of the wind stress, the latter mechanism being significant mainly near the equator. We have constructed a simplified wind field over the Bay (Figure 5) by averaging in space the amplitudes of the annual and semi-annual harmonics of the climatological²³ wind stress. The annual and semi-annual harmonics were calculated using a least-square fit to monthly-mean wind stress²³.

The wind stress shown in Figure 5 was applied only over the region north of 9°N. South of this latitude the stress was decreased linearly to zero at 5°N. This tapering of wind stress introduces curl in the region 5–9°N. In the discussion below, we examine only the region north of 10°N. Shown in Figure 6 are velocities at 35 m, i.e. at a depth where Ekman velocity does not dominate the flow field.

The wind-field shown in Figure 5 essentially consists of two regimes. The first, made of southwesterly winds is stronger and starts building up during April. In response, a Kelvin wave pulse is generated along the eastern boundary (Figure 6 b). As the winds strengthen, the velocity field associated with the Kelvin wave strengthens. Kelvin waves can radiate Rossby waves offshore.

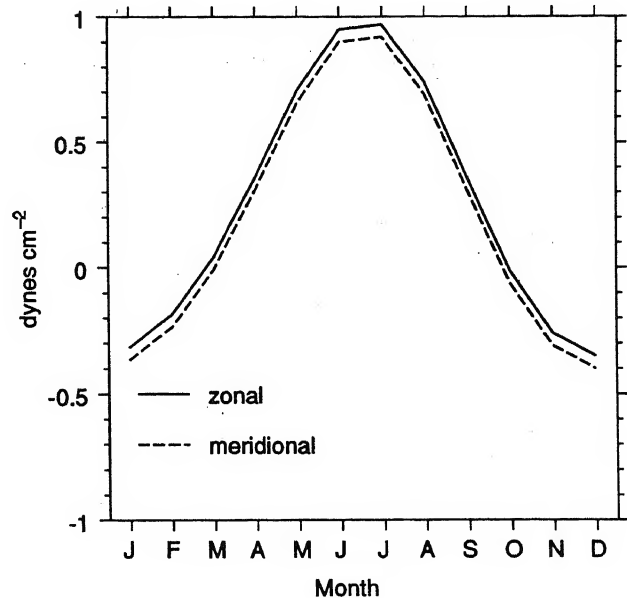


Figure 5. Annual cycle of zonal (full line) and meridional components (dashed line) of simplified spatially uniform wind stress.

By June (Figure 6 c), the Rossby wave radiated by the Kelvin wave can be seen as far west as 90°E. In addition, a patch of westward velocity can be seen west of about 87°E between 12 and 15°N. For the wind field used here, the only mechanism that can give rise to such a patch is Ekman suction due to β -effect. The upwelling favourable winds along the western boundary drive a poleward EICC there. This current weakens during July, although the wind stress peaks then. This is caused by the arrival of the downwelling Kelvin wave from the eastern boundary (Figure 6 c). The downwelling Kelvin wave is modified by the local upwelling favourable winds. In the northern part of the coast the downwelling wave dominates; as one moves southward, however, local forcing overwhelms it.

The southwesterly winds weaken during August. Seeking equilibrium with weakening winds, the velocity associated with the Kelvin wave at the eastern boundary falls to zero, and the flow then turns southward (Figure 6 d, e). This starts an upwelling phase of the Kelvin wave along the eastern boundary of the Bay. The southwesterlies withdraw during September and October, and the Kelvin wave along the eastern boundary has southward velocity along the entire coast (Figure 6 e).

During August the northward velocity associated with the Rossby wave radiated by the downwelling Kelvin wave (April–July) covers the entire Bay (Figure 6 d). As the velocity associated with the Kelvin wave at the eastern boundary turns southward, the velocity associated with the westward moving Rossby wave also turns southward (Figure 6 e). A region of weak flow separates

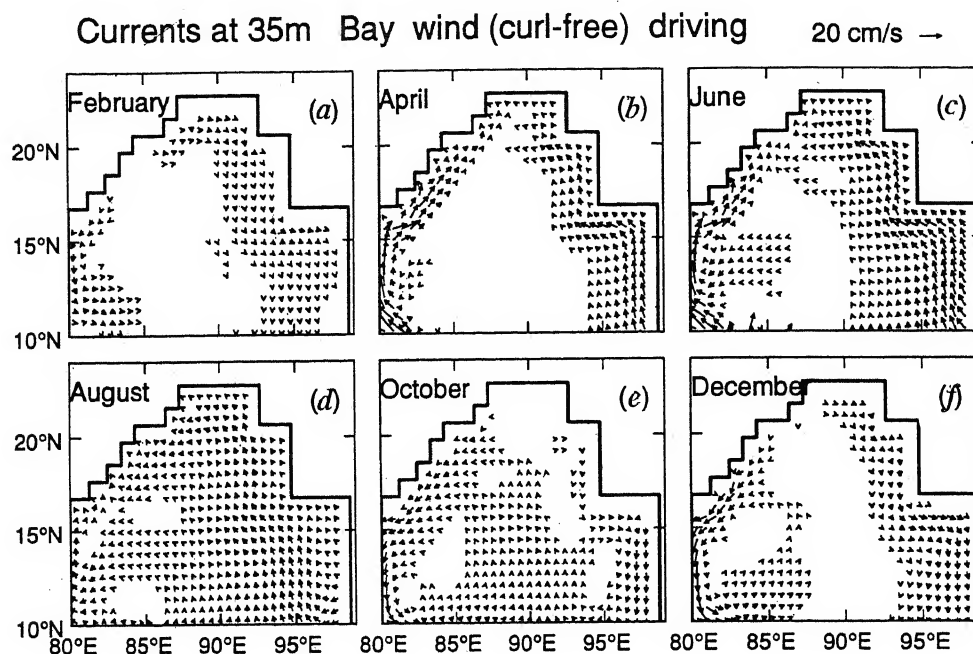


Figure 6. Currents at 35 m for the run with wind stress shown in Figure 5. The wind stress was applied only over the Bay of Bengal. A current vector was plotted only when the magnitude exceeded 2 cm s^{-1} .

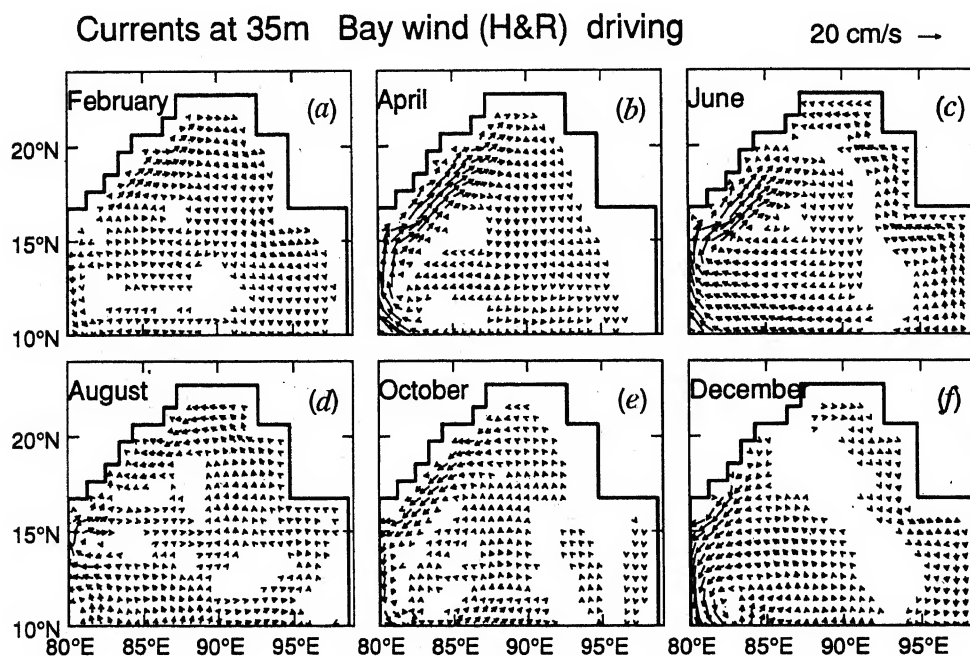


Figure 7. Same as Figure 6 but for the run with H & R winds applied only over the Bay.

(Figure 6 *e*) the regions of northward and southward velocities; the latter is seen only close to the coast.

The decrease of southwesterly winds also affects the flow along the western boundary: the locally driven

poleward current weakens (Figure 6 *d*). As the southwesterly winds collapse, adjustment to the new wind field leads to the southward current (Figure 6 *e*), a process highlighted by MKM. Another mechanism that adds

to the southward current is the reflection of the leading edge of the Rossby wave. Upon reflection this wave produces a narrow band of southward velocity. This reflected Rossby wave has a smaller wavelength.

The northeasterly winds are most intense during November–December. In response, an upwelling Kelvin wave forms along the eastern boundary. It is weaker than the downwelling Kelvin wave during April–July (compare Figure 6f with Figure 6c) because the wind stress is weaker.

During November, the Rossby wave radiated by the downwelling Kelvin wave of the summer monsoon is still present in the central Bay. This is seen as the westward migration of a patch with northward velocity, its reflection from the western boundary and the resulting southward EICC. In the eastern half of the Bay the Rossby wave pulse radiated by the upwelling Kelvin wave is present (Figure 6f). However, there is no indication of this Rossby wave crossing the Bay to have an impact on the western boundary. This is because the Rossby wave packet is weak and gets dissipated by the time it reaches the central Bay. Along the western boundary the northeasterly winds contribute to an equatorward flow during November–January (Figure 6f). By March the winds collapse; adjustment to the new winds leads to the formation of the poleward EICC.

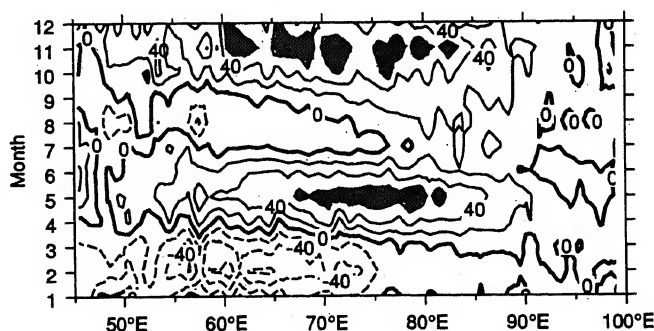
Comparing Figures 6 and 2 we see that the overall pattern of near surface circulation has been reproduced well in this run with simplified winds. The process that was most active in this run was the generation of Kelvin wave pulses along the eastern boundary of the Bay. An important difference between this run and the one discussed in section 3 is that the circulation is weak in the former, implying that other mechanisms must be present to enhance the circulation.

Circulation due to winds over the Bay

In this experiment the model was forced by the annual cycle of H & R wind stress only over the Bay of Bengal. The purpose of this experiment was to examine the circulation in the Bay in the absence of equatorial wind forcing. An important difference between the run in the previous sub-section and the present run is that now Ekman suction from curl of wind stress is present. The simulated currents at 35 m are shown in Figure 7. Below we discuss the changes seen in this figure from the circulation described in section 5.1

During March–April, the main difference from the previous run is that there is a well developed anticyclonic gyre with a poleward EICC (Figure 7b). Because of the gyre, the velocity field in the open Bay is stronger (compare Figure 6b with Figure 7b). The primary cause of the difference between these figures is the contribution from wind stress curl. The currents along the eastern Bay are prominent in the case of curl-free driving due to the alongshore winds.

(a) Ship-drift U-component 2°S–2°N



(b) Model U-component Equator

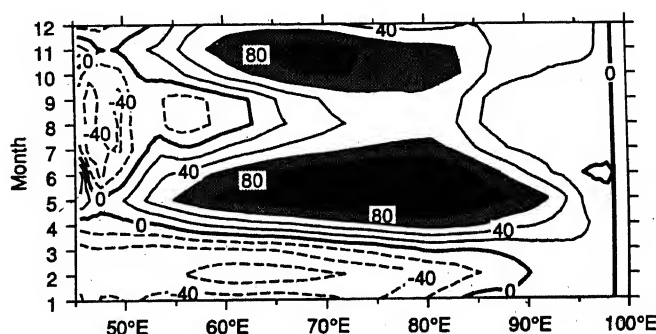


Figure 8. Time longitude sections of zonal velocity (i) in the equatorial region (averaged between 2°S and 2°N) from the ship-drifts and (ii) along the equator from the model. Thick line represents zero contour and dotted contours represent westward flow. Eastward velocity greater than 60 cm s^{-1} is shaded to show the equatorial jets.

During November–January, the southward EICC is stronger in the present run (compare Figure 6f with Figure 7f). The principal cause behind this change is a patch of wind stress curl which forms over the region off Sri Lanka during October. The northward flow in the open Bay seen in the earlier experiment was the result of the Rossby wave radiated from the eastern boundary. Now, in the presence of wind stress curl, the contribution to the velocity field from that Rossby wave is increased due to the contribution from wind stress curl. The net result is a strengthening of the equatorward EICC.

Thus, the presence of wind stress curl strengthens the EICC by generating two gyres: an anticyclonic gyre during March–May in the Bay and a cyclonic gyre off the coast of southern India during the winter.

Circulation due to winds over the equatorial Indian Ocean

The source of all equatorial effects in the Bay is the equatorial Kelvin wave. To ensure that equatorial effects are properly accounted for, it is necessary that equatorial circulation be correctly simulated by the model.

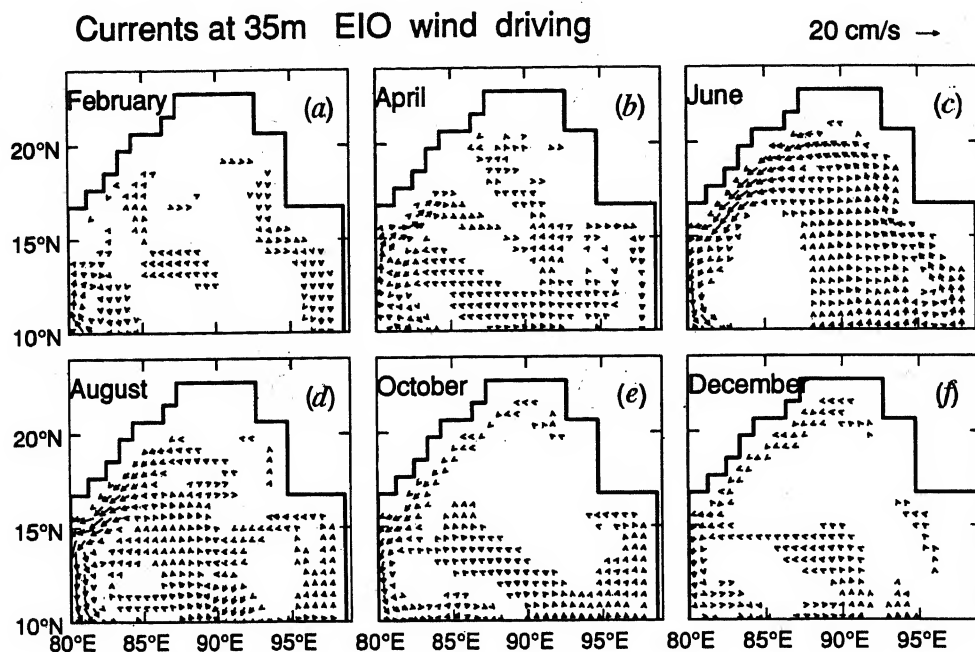


Figure 9. Currents at 35 m due to equatorial processes. This is calculated by subtracting the model result with winds (H & R) only over the Bay from the model result with winds (H & R) over the entire model domain. A current vector was plotted only when the magnitude exceeded 2 cm s^{-1} .

This can be seen in Figure 8 *a, b*, which shows the zonal component of monthly mean ship-drifts¹¹ and zonal velocity from the model. There is good resemblance between Figure 8 *a* and *b*, implying that equatorial circulation has been reasonably well reproduced. In particular, during April–May and October–November strong eastward jets similar to those first noted by Wyrtki³² occur in the model. Note that MKM were unable to simulate the fall jet.

To study the impact of equatorial processes on the Bay of Bengal circulation, the velocity field in the run with winds only over the Bay was subtracted from that with winds over the entire model domain. The resulting velocity field is shown in Figure 9.

The velocity field in the Bay associated with equatorial forcing is weak (Figure 9). The most significant result of this driving is an equatorward current during the summer monsoon. This current is opposite in direction to those seen in the earlier two experiments (compare Figure 9 *c, d* with Figure 7 *c, d* and Figure 6 *c, d*).

In brief, the numerical experiments carried out here show that we can understand the annual cycle of circulation in the Bay in terms of the following: (i) Two Kelvin wave pulses generated by alongshore winds in the coastal region of the Bay, mainly its eastern boundary. (ii) Circulation set up by wind stress curl over the Bay; primarily an anticyclonic gyre during March–May and a cyclonic gyre in the southwestern Bay during

October–December. (iii) A weak circulation set up by equatorial forcing, whose main effect is to reduce the strength of the poleward current during the summer monsoon.

6. Conclusions

The results of numerical experiments discussed above show the usefulness of the OGCM in simulating and understanding the upper-layer circulation in the Bay of Bengal. The model reproduces most of the features seen in the available data sets. The results of the numerical experiments allow us to construct the following hierarchy of processes dominating the circulation in the Bay:

- i) *Coastal Kelvin wave pulses generated along the boundary region, primarily the eastern boundary*

The more important of the two pulses is the downwelling pulse generated with the onset of the south-westerly winds. Its impact on the coastal current is most important in the north during the summer monsoon. The second pulse, an upwelling event, occurs during the winter monsoon and it generates a poleward EICC during March. Both these pulses contribute towards circulation in the Bay by radiating Rossby waves. However, it is necessary to invoke

other mechanisms to explain all the features of circulation.

ii) *Rossby waves generated by Ekman suction over the Bay*

The main function of this mechanism is to strengthen the EICC by generating an anticyclonic gyre during March–May and a cyclonic gyre during the winter monsoon.

iii) *Effects derived from the equatorial region*

The most significant effect of this forcing is an equatorward current off the east coast of India during the summer monsoon. This current is opposite to that driven by the winds over the Bay.

It is clear that more modelling studies are required, for instance, to improve the simulation of the currents during the summer monsoon. Above all, a well planned observational programme is a must for further progress in understanding the dynamics and thermodynamics of the Bay of Bengal and its coupling with the monsoon. Although theoretical studies suggest that low-frequency Rossby and Kelvin waves are crucial to the circulation, their presence has not been established in the field. The impact of freshwater driving and the nature of space-time variation of the stratification of the Bay also needs to be elucidated with the help of more observations. None of the model studies so far have taken into account the freshwater input into the Bay explicitly. In future modelling studies, the important problem of understanding the role of buoyancy driving in the circulation of the Bay will be addressed.

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Comparison of three different tests for detection of cucumber mosaic cucumovirus in banana (*Musa paradisiaca*)

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Direct ELISAs like double antibody sandwich (DAS)-ELISA, simplified rapid direct antigen coating (SRDAC)-ELISA and indirect form of DAC-ELISA for detection of cucumber mosaic cucumovirus (CMV) causing infectious chlorosis of banana in leaf extracts and pseudostem sap exudates and purified virus diluted with antigen buffer or healthy banana leaf sap were compared. The sensitivity levels of three ELISA tests were similar and the virus was detected up to 10^{-7} dilution with leaf extract, 1 μ l/well with sap exudate, 20 ng/well with purified CMV and 100 ng/well with purified virus diluted with healthy banana leaf extract. Of three forms of ELISA compared, DAC-ELISA was further evaluated with detection of infectious virus by local lesion bioassay on cowpea (*Vigna unguiculata*) and by double stranded RNA (dsRNA) analysis. The banana leaf and pseudostem sap exudate samples that were positive in DAC-ELISA were also positive by other two tests. Collection of pseudostem sap exudates by pin pricking from test plants and detection of virus by DAC-ELISA in them appears ideal for large-scale testing of banana plants.

THE banana (*Musa paradisiaca*) is one of the world's most important tropical fruit crops. It is grown in all types of tropical agricultural systems¹. Bananas are propagated vegetatively through suckers. Viruses as systemic pathogens are readily disseminated in vegetative propagation material and usually cause serious problems in crops propagated in this way.

The causal viruses of bunchy top (banana bunchy top virus-BBTV), infectious chlorosis (cucumber mosaic cucumovirus-CMV), bract mosaic (banana bract mosaic potyvirus-BBMV), streak (banana streak badnavirus-BSV) and tobacco mosaic virus¹⁻⁴ diseases of banana are vertically transmitted through planting suckers.

Four viruses are known to naturally infect banana in East and West Godavari districts of Andhra Pradesh state in India (personal communication, Krishna Prasadji, Banana Research Station, APAU, Kovvur). The field identification of diseases caused by CMV, BBMV and BSV based on the symptoms in banana is difficult as they induce similar symptoms (chlorotic streaks) at certain stages of disease development.

The infectious chlorosis disease caused by CMV has been reported from India, Australia, Greece and Morocco⁵⁻¹⁴. Recently, a virus causing severe chloro-

sis/mosaic disease of banana in Uttar Pradesh, India was identified as a strain of CMV using slot-blot hybridization with nucleic acid probe of CMV-P genome¹⁵. Currently, the recommended strategy for control of CMV in banana is to identify virus-infected plants, remove the diseased plants and replant with virus-free banana plants¹. In order for this approach to be successful, it is necessary to have sensitive, rapid and reliable indexing tests for the detection of CMV from banana plants. Hansen and Wick¹⁶ reviewed the various biological, immunological and biochemical tests used for detection and identification of plant viruses. Of these, ELISA-based tests have been widely used because of their simplicity, economy and adaptability for large-scale testing of suspected plant samples.

In view of potential importance of CMV in banana¹⁷, we evaluated three ELISA procedures and compared one of them with bioassay and double stranded (ds) RNA analysis for detection of CMV in banana leaf extracts and pseudostem sap exudates collected by pin pricking.

CMV causing infectious chlorosis in banana plants growing under natural conditions in Andhra Pradesh served as the source of primary inoculum. The virus culture was initially established on cowpea and single lesion isolate was subsequently propagated on tobacco by sap inoculation and on banana by injecting the purified virus¹⁸. Virus-free banana plants were propagated by transplanting virus-free suckers indexed by DAC-ELISA. The virus was purified from virus-infected tobacco leaves harvested twenty days after sap inoculation essentially by following the procedure of Walkey¹⁹. The concentration of purified virus was determined by considering 5 OD units = 1 mg (ref. 20).

Polyclonal antiserum prepared previously to CMV-banana isolate was used in this study¹⁸. Immunoglobulins (IgG) from crude CMV-banana antiserum for direct ELISA procedures were purified by sodium sulphate precipitation followed by dialysis as described by Rajeswari *et al.*²¹. Alkaline phosphatase (Sigma) was conjugated to CMV IgG and goat antirabbit antibodies (Gibco, USA) by one step glutaraldehyde method²².

The composition of buffers and plate-washing procedures were similar to those of Clark and Adams²². The volumes of reactants added to each step unless otherwise stated were 200 μ l/well. All ELISA incubation periods prior to substrate addition were 90 min at 37°C in a humid box. ELISA plates with flat bottom wells (Laxbro, Pune) were used in all procedures.

The antigen samples for simultaneous testing used were banana pseudostem sap exudate collected separately from healthy and CMV-infected banana by pin pricking the pseudostem (10, 20, 50, 100 μ l/ml), banana leaf sap (10^{-1} – 10^{-8} dilution), purified CMV-banana (0.1–2.0 μ g/ml). For direct antigen coating procedures (DAC, SRDAC) the antigens were extracted (1 g/9 ml buffer) and diluted in 0.05 M carbonate buffer, pH 9.6

Table 1. Detection of CMV causing infectious chlorosis of banana by different forms of ELISA

Type of antigen	Antigen concentration ^a	Direct form		Indirect form
		DAS-ELISA ^b	SRDAC-ELISA ^c	DAC-ELISA ^d
CMV-infected banana leaf sap	10 ⁻¹	2.80 ^e	1.93	3.0
	10 ⁻²	2.62	1.85	3.0
	10 ⁻³	2.02	1.53	2.20
	10 ⁻⁴	1.18	1.12	2.01
	10 ⁻⁵	0.94	0.85	1.13
	10 ⁻⁶	0.82	0.73	1.00
	10 ⁻⁷	0.31	0.28	0.33
	10 ⁻⁸	0.09	0.07	0.10
Healthy banana leaf sap	10 ⁻¹	0.06	0.07	0.05
	10 ⁻²	0.02	0.03	0.03
CMV-infected banana pseudostem sap exudate (µl/ml)	100	2.84	2.20	3.01
	50	2.71	1.91	3.0
	20	2.04	1.50	2.32
	10	1.25	0.94	1.52
	5	0.41	0.23	0.43
	2	0.04	0.05	0.02
Healthy pseudostem sap exudate (µl/ml)	100	0.09	0.10	0.07
	50	0.08	0.05	0.06
Purified CMV (µg/ml) diluted with antigen extraction buffer	2.0	2.53	2.71	2.75
	1.0	2.09	1.98	2.14
	0.5	1.14	1.03	1.20
	0.1	0.51	0.87	0.40
	0.01	0.04	0.03	0.06
Purified CMV diluted with healthy banana leaf sap (µg/ml) extracted with antigen extraction buffer	2.0	0.97	0.95	1.21
	1.0	0.63	0.70	1.13
	0.5	0.32	0.42	0.64
	0.1	0.04	0.02	0.07

^aAntigens (Sap, purified virus and pseudostem sap exudate) diluted in PBS-TO containing DIECA for DAS-ELISA and in carbonate buffer containing DIECA for DAC and SRDAC-ELISA; ^b,^cALP labelled CMV-B antibodies used at 1:500; ^dALP labelled goat antirabbit antibodies used at 1:1000;

^eValues are an average absorbance A₄₀₅ of three wells recorded after 90 min of adding substrate.

containing 0.01 M DIECA. Whereas for DAS-ELISA they were extracted and diluted in PBS-TO containing 0.01 M DIECA. The antigen dilutions in each procedure were replicated in three wells in each of the experiments.

Antiserum and enzyme-conjugated IgG were diluted in PBS-TO. *p*-Nitrophenyl phosphate (PNP) prepared just before use at 0.5 mg/ml diethanolamine buffer, pH 9.8 was used as a substrate, NaOH (3M) at 50 µl/well used to terminate the reactions.

DAS-ELISA described by Clark and Barjoseph²³ was followed. CMV-banana IgG at 1:500 in carbonate buffer, pH 9.6 was added to the plates as trapping antibodies. Antigen samples prepared in PBS-TO were added after washing the plate, followed by alkaline phosphatase labelled CMV-IgG at 1:500 dilution. After washing, the substrate was added and incubated at room temperature for 90 min. Absorbance readings (A₄₀₅) were recorded with Bio-Tek Ceres 900 ELISA reader over buffer controls. Readings twice those of healthy were considered as positive.

The procedure for SRDAC-ELISA was similar to DAS, but here the plates were directly coated with antigen samples prepared in carbonate buffer, pH 9.6 (ref. 24). The trapped antigens were detected by alkaline phosphatase labelled CMV-IgG as described above.

For DAC-ELISA, the plates were directly coated with antigen samples prepared in carbonate buffer, pH 9.6 (ref. 25). Crude CMV-banana polyclonal antiserum at 1:500 dilution in PBS-TO was added after washing. Alkaline phosphatase labelled goat antirabbit antibodies at 1:1000 in PBS-TO were added to the plate to detect the antigen-antibody reaction. The plate was incubated with *p*-nitrophenyl phosphate for 90 min at room temperature. The absorbance values were recorded as described under DAS-ELISA.

The virus-infected banana leaf samples from different plants were separately ground (1 g/9 ml) in inoculation buffer (0.01 M KPO₄ buffer, pH 7.2 containing 0.2% 2-mercaptoethanol) and sap inoculated to carborundum powder (600 mesh) dusted *Vigna unguiculata* plants and observed for development of local lesions. Pseudostem

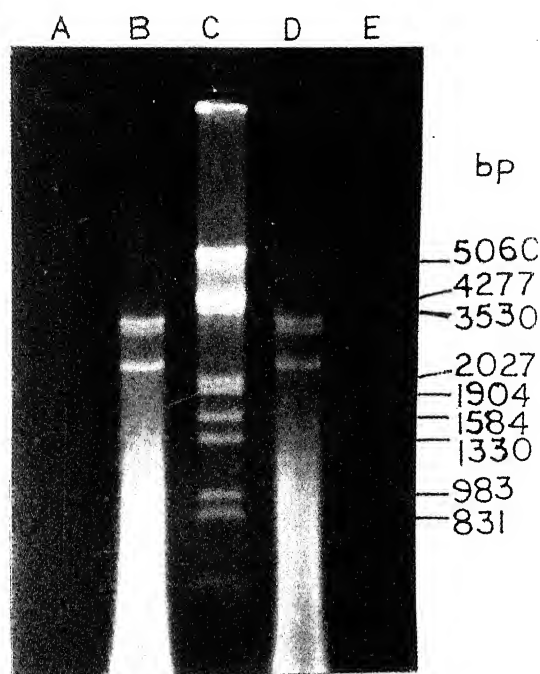


Figure 1. dsRNA analysis of CMV-Banana isolate in 2% agarose gel. Lane A, dsRNA isolated from healthy banana pseudostem sap exudate; B, dsRNA isolated from CMV-infected banana pseudostem sap exudate; C, λ DNA/*Eco*RI & *Hind*III digested marker (Mr values are shown on right side); D, dsRNA isolated from CMV-infected banana leaf sap; E, dsRNA isolated from healthy banana leaf sap.

Table 2. Evaluation of DAC-ELISA for detection of CMV in banana leaves and pseudostem sap exudate

Nature of banana plant	Leaves	Pseudostem sap sap exudate
CMV-infected banana		
1	3.02 ^a	3.55
2	2.97	3.48
3	3.11	3.31
4	3.17	3.27
5	3.01	3.52
6	2.92	3.47
7	2.85	3.25
8	3.07	3.29
9	3.05	3.40
10	2.90	3.38
11	3.01	3.37
12	3.0	3.19
Healthy banana		
1	0.07	0.05
2	0.07	0.02
3	0.03	0.07
4	0.10	0.11
5	0.08	0.09
6	0.12	0.08
7	0.04	0.07
8	0.11	0.03
9	0.09	0.07
10	0.05	0.04

^aFigures represent an average of three A₄₀₅ readings.

sap exudate was separately collected by pin pricking from different infected plants and diluted with inoculation buffer (500 μ l/ml). This sap was inoculated to abrasive dusted *V. unguiculata* plants.

The dsRNA was isolated from CMV-infected banana leaves and pseudostem sap exudates by lithium chloride fractionation method of Diaz-Ruiz and Kaper²⁶ and analysed by 2% agarose gel electrophoresis²⁷.

The concentrations of crude antiserum, IgG, enzyme-labelled IgG used in the present study were chosen based on preliminary experiments. Further dilutions of these reagents resulted in weak reactions. The virus was detectable at 10^{-7} dilution of banana leaf sap, 5 μ l/ml (1 μ l/well) of pseudostem sap exudate, 100 ng/ml (20 ng/well) of purified CMV-banana and 500 ng/ml (100 ng/ml) of purified CMV-banana diluted with healthy banana leaf sap in all three tests (Table 1). However, the absorbance values were marginally higher in DAC-ELISA when compared to other two tests. Dilutions of the purified virus with healthy banana leaf extract resulted in decreased sensitivity of virus detection by about five times. Among three forms of ELISA, the sensitivity of DAC-ELISA was compared with local lesion bioassay and dsRNA analysis tests for detection of CMV in banana (Table 2). The banana leaf samples and pseudostem sap exudates that were positive by DAC-ELISA were also positive by the other two tests. In the bio-assay test, cowpea plants inoculated with both infected banana leaf extract and pseudostem sap exudate produced necrotic local lesions after three days of sap inoculation [23 to 34 lesions/primary leaf]. The dsRNA extracted from both infected leaf and pseudostem sap exudate was resolved into 3 bands (Figure 1) with Mr of RNA 1–2.56, RNA 2–2.24 and RNA 3– 1.66×10^6 d. No such bands were observed in the corresponding healthy plant samples.

Biological, immunological and molecular techniques are used for diagnosis of CMV-caused diseases¹¹. The choice of these techniques is based on expertise and laboratory facilities available, specificity, sensitivity, cost and time factor. Among serological techniques agargel diffusion and ELISA based ones were used for detection of CMV in various plant samples.

In the present investigation, we evaluated both direct (DAS-ELISA and SRDAC-ELISA) and indirect (DAC-ELISA) forms of ELISA for detection of CMV in banana. Sampling from banana by way of collection of pseudostem sap exudate by pinpricking appears simple and ideal in large-scale testing of banana plants/planting suckers (sometimes without leaves) when compared to collection of leaf samples and preparation of extracts from them.

In the present studies the virus was detectable at 10^{-7} dilution of banana leaf sap, 5 μ l/ml of pseudostem sap exudate and 100 ng/ml of purified CMV-banana in all three ELISA tests. During the extraction of banana

leaves for CMV indexing, samples that oxidize despite the presence of reducing agents can produce false positive reactions in ELISA¹¹. These reactions can be minimized by thorough washing of the mucilaginous antigen samples from the wells of the plate before adding the next reagent.

Eyen though there is no significant difference in the sensitivity levels of virus detection with three tests, the reagents and time required to carry are varied. DAC-ELISA is economic, requires about 5–6 h and is more versatile as the test can be automated and commercialized for application to crops like banana.

In laboratories with minimal facilities, more glasshouse and time (3–4 days) are not the criteria, detection of CMV in banana by bioassay on local lesion hosts like cowpea or green gram (*Phaseolus aureus*) appears suitable routine practice. Further, the other four viruses known to infect banana did not infect these local lesion hosts and thus bioassay is useful for distinguishing CMV from other viruses of banana.

The dsRNA analysis has been used as one of the criteria for identifying the various isolates of CMV in Australia¹². The application of this technique requires more time (2–3 days), expertise and expensive laboratory facilities. Moreover it is suitable for detecting the virus in a small number of samples. The samples that were positive by DAC-ELISA are also positive by the other two tests (Table 2). Finally we conclude that DAC-ELISA is a suitable test for routine application in large-scale testing of banana encountered in plant quarantine, in planting material (suckers) certification programmes and in banana field surveys.

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Thin layer problem in geoelectrics

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Inferences in the influence of a thin conductive layer on the controlled source audiofrequency magnetotelluric (CSAMT) Cagniard response as compared to magnetotelluric (MT) soundings are studied. For this purpose, H_r and E_ϕ components are computed due to a horizontal electric dipole (HED) over a layered media without imposing farfield or nearfield conditions for the CSAMT method. The computation is carried out in a cylindrical coordinate system using Hankel transform of Bessel functions of order zero and unity with a 100 point digital filter.

The root mean square (RMS) deviations between the CSAMT response with and without thin conductive layer, in the frequency ranges of 0.125 Hz to 4096 Hz are computed. A similar scheme of computation is implemented for the MT response. This difference is uniformly higher for the CSAMT within the range of parameters investigated and hence the CSAMT method is preferable over the MT method for delineation of the thin intermediate conductive layer. Conversely, if a lesser influence of the thin conductive layer is desired, MT soundings are preferred over CSAMT studies.

RESEARCH COMMUNICATIONS

THE influence of a thin conductive layer has been studied in electromagnetic prospecting by several authors¹⁻¹⁵. Molochnov¹⁶ studied the influence of a thin conducting layer on the field components of a vertical magnetic dipole (VMD) source in terms of tabulated functions so that the maximum effect can be computed for any chosen frequency. The present study is aimed at assessing the influence of thin conductive layer on CSAMT Cagniard response as compared to MT. This study acquires added significance in view of the presence of conductive thin layers (red bole) and intra- and infratrappean sedimentary sequences in the vast Deccan flood basalt region which is coming into sharp focus for two reasons, namely, neotectonic activity and energy resource potential of the lithounits below the Deccan trap covered terrain.

The components of the electromagnetic field on the surface of a multilayered earth for a horizontal electric dipole (HED) are available in literature¹⁷. Over a multilayered earth for far field conditions ($|k| \gg 1$ where k is the wave number given by $k = \sqrt{j\omega\mu}$), the apparent resistivity can be obtained as

$$\rho_a = \frac{1}{\omega\mu} |Z_n|^2, \quad (1)$$

where Z_n is the ratio of electric and magnetic field components.

The apparent resistivity for the MT method over a multilayered earth¹⁸ can be obtained through equation (1), where the impedance Z_n is given by

$$Z_n = \frac{\omega\mu}{k_1} \cot h \left\{ -ik_1 h_1 + \cot h^{-1} \left[\frac{k_1}{k_2} \cot h \left(-ik_2 h_2 + \cot h^{-1} \left\{ \frac{k_2}{k_3} \cot h \left(-ik_3 h_3 + \dots + \cot h^{-1} \left(\frac{k_{n-2}}{k_{n-1}} \cot h \left(-ik_{n-1} h_{n-1} + \cot h^{-1} \frac{k_{n-1}}{k_n} \right) \right) \right\} \right) \right] \right] \right\}. \quad (2)$$

Thus, in the far field conditions, the CSAMT apparent resistivity fully coincides with the MT Cagniard resistivity. However, a marked difference exists when the condition of $|k| \gg 1$ is not valid¹⁹⁻²¹.

We computed two categories of Cagniard CSAMT responses as a function of frequency using the corresponding equations (expressions 2.191, 2.209 and 2.210) (ref. 17) for a multilayered earth in order to investigate the effect of a thin conductive layer on the CSAMT response. Category I corresponds to two-layer geoelectric model for different ρ_2/ρ_1 and r/h_1 values, where r is the transmitter receiver separation and ρ_1 , ρ_2 and h_1 are the resistivities of the first and second layers and the thickness of the top layer respectively. Category II corresponds to geoelectric models with a thin layer of constant thickness (1 m and 2 m) and resistivities

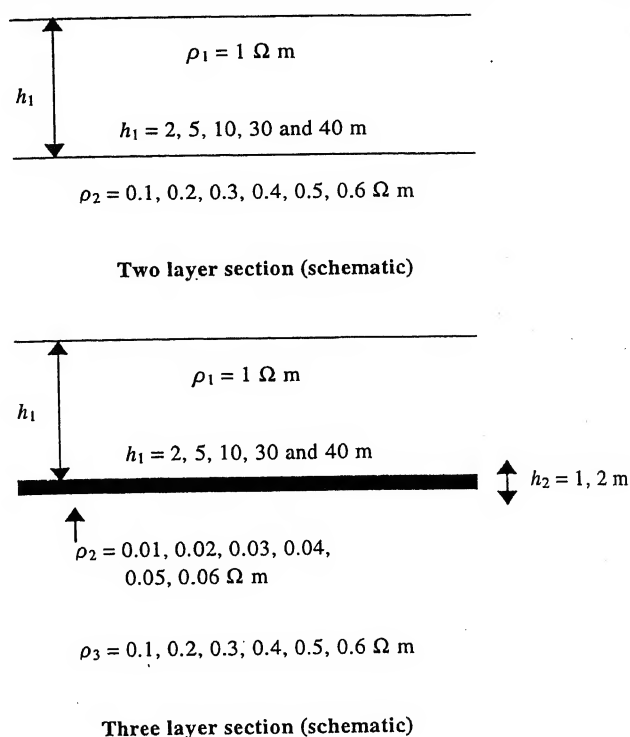


Figure 1. Geoelectrical models.

(0.04 ohm m) with varying depth of burial introduced into the two-layer system (chosen in Category I). The details of these models are given in Figure 1. The MT response curves were computed using equations (1) and (2) for the same geoelectrical models.

The scheme of computation developed for this purpose first computes the H_r and E_ϕ components due to a HED source over layered media without imposing either the farfield or the nearfield conditions and then computes the Cagniard resistivities and RMS difference²² between CSAMT response curves with and without the thin layer, in the entire measurement range of frequencies. The electromagnetic field of HED was computed in a cylindrical coordinate system using Hankel transforms of Bessel functions of order zero and unity with a 100 point digital filter²³.

A similar computational scheme implements the procedure for MT response.

Figures 2 and 3 show examples of four pairs of response curves for the CSAMT and MT respectively. The solid curves a, c, e and g in Figure 2 are two layer cases with resistivities $\rho_1 = 1.0$ ohm m and $\rho_2 = 0.66$ ohm m and $h_1 = 2, 5, 10$ and 20 m in this order (category I). The broken curves (b, d, f and h) shown in Figure 2 involve a thin conducting layer of resistivity 0.04 ohm m and thickness 1 m at depths 2, 5, 10 and 20 m in this order (category II). A similar representation has been used for the MT responses in Figure 3.

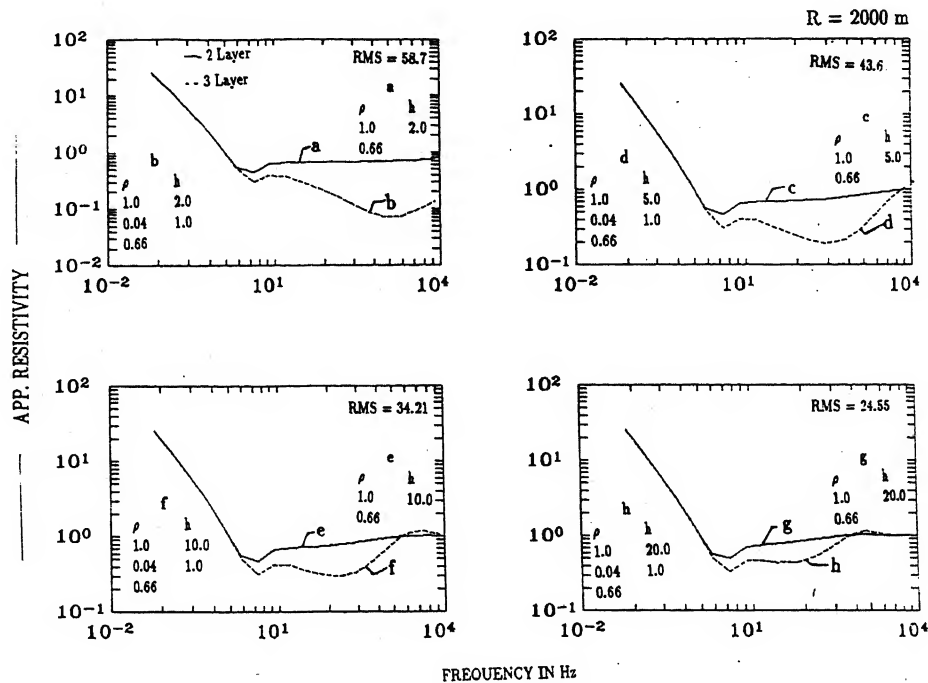


Figure 2. CSAMT response without and with the thin conductive layer.

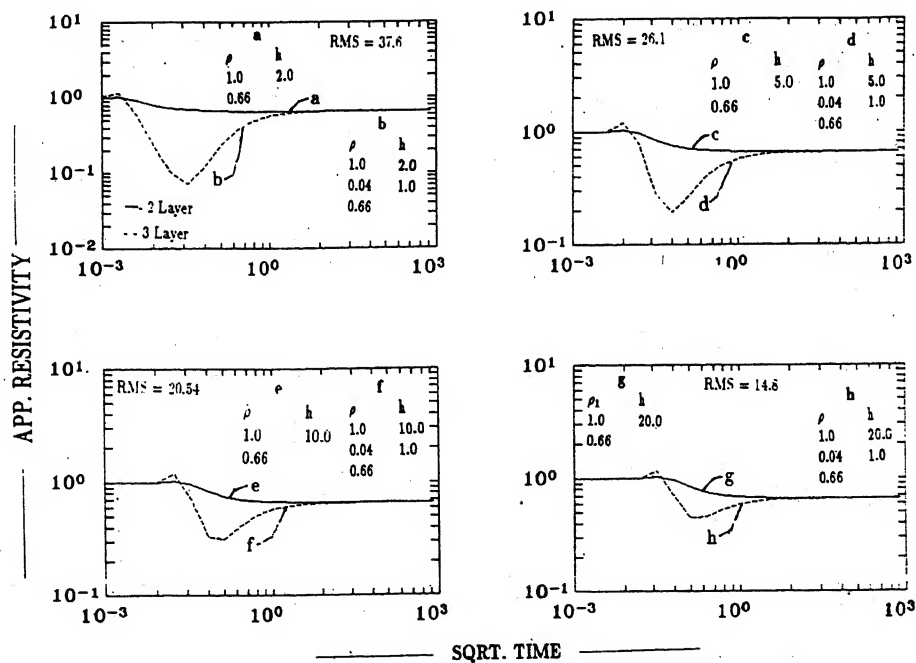


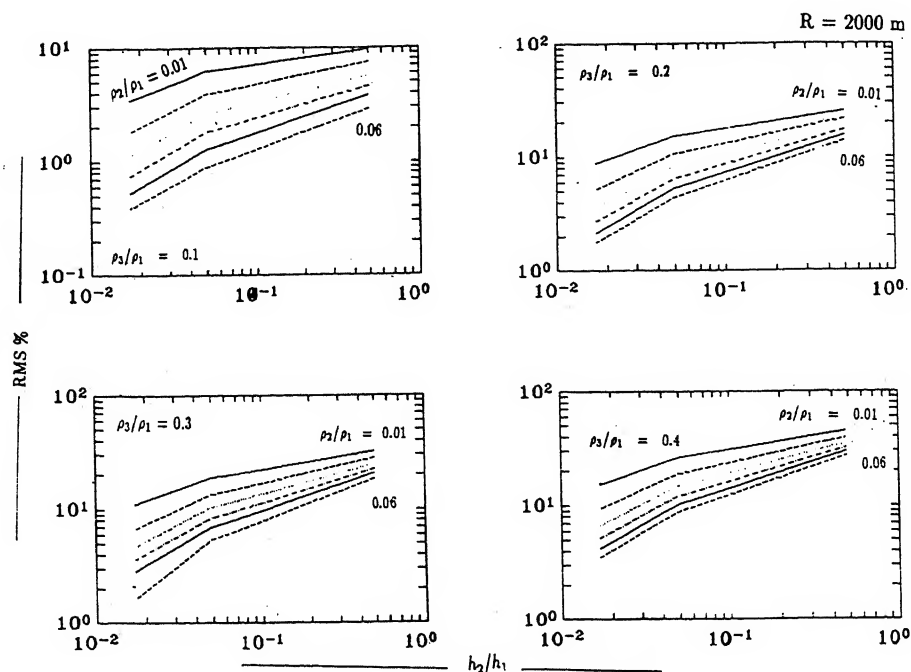
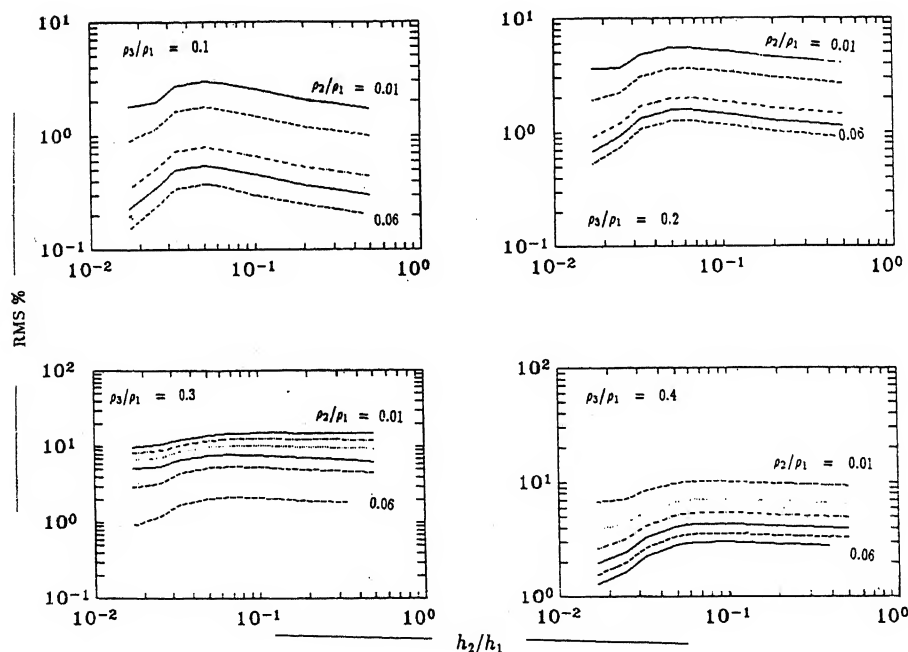
Figure 3. MT response without and with the thin conductive layer.

The normalized non-dimensional presentation allows one to understand the effects for multitude of geoelectric truths from the response functions thus computed.

We can note (from Figure 2), as is expected, that the RMS difference between the CSAMT response with and

without the conducting layer progressively diminishes as the depth to the thin conductive layer increases.

The relationship of RMS difference with h_2/h_1 for various ρ_2/ρ_1 ratios for a particular ρ_3/ρ_1 value in the CSAMT and MT is shown in Figures 4 and 5 respec-

Figure 4. Relationship between h_2/h_1 and RMS error % for CSAMT data.Figure 5. Relationship between h_2/h_1 and RMS error % for MT data.

tively. We thus obtained the combination of h_2/h_1 , ρ_2/ρ_1 and ρ_3/ρ_1 for any chosen RMS value.

Figure 6 shows a comparison of the RMS deviation in percent for different thickness ratios in the CSAMT and MT techniques. The RMS deviation is consistently

higher for the CSAMT technique (compared to MT) within the range of parameters investigated and hence the delineation of the thin conducting layer as a separate entity is better in CSAMT when compared to MT. However, in case lesser influence of the intermediate con-

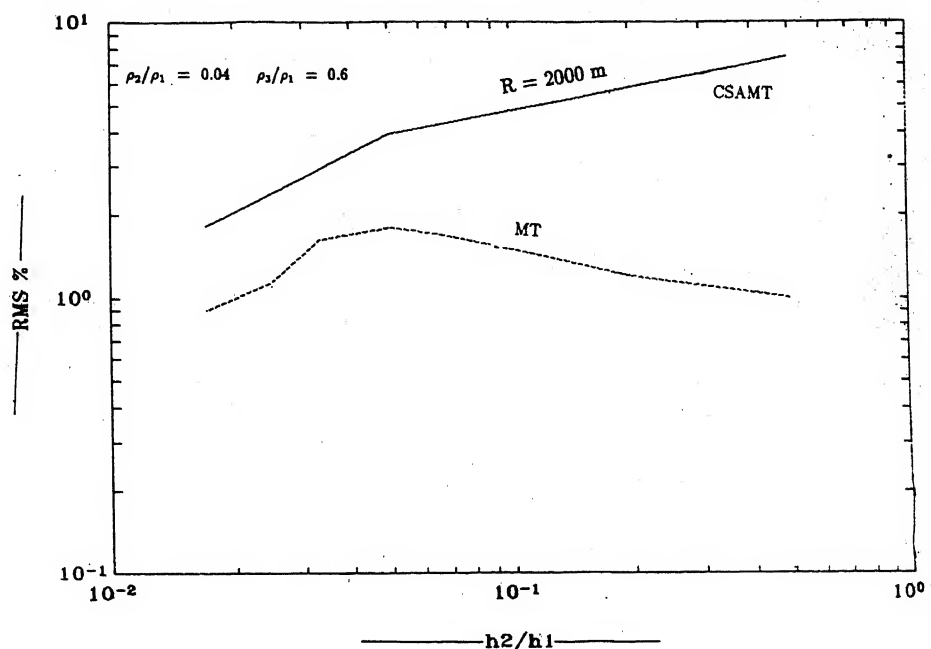


Figure 6. Comparison of RMS error in CSAMT and MT.

ducting layer is desired, MT soundings are preferable over CSAMT studies. In general, it appears that the influence of a thin conductive layer increases, with an increasing h_2/h_1 for given resistivity contrast in the CSAMT. On the other hand it is interesting to observe that a sort of a 'tuning effect' with respective h_2/h_1 is seen in the MT technique for certain resistivity contrasts, which implies that within a range of h_2/h_1 (0.03 to 0.05 in Figure 6) the influence of thin conductive layer is prominent in the MT technique. However, this tuning effect depends on the resistivity contrast also. For large values of h_2/h_1 , the CSAMT sounding appears to be more suitable than the MT technique, when the study is aimed at detecting the intermediate conductive layer.

Thus, it is desirable to explore the potential of the CSAMT technique for the effective detection of sedimentary units intermediate conductive layers (intratrapeans, redbole vesicular trap horizons, weak zones, etc.) in the Deccan trap regions of the country.

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Geohydrology of springs in a mountain watershed: The need for problem solving research

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Spring discharge is controlled by rainfall, land use, vegetation, grazing incidence and geomorphology of the recharge zone in a mountain watershed in Garhwal Himalaya. The decline in discharge was found low (36.4%) in a fracture/joint/colluvial related spring with SE aspect, a mix of agriculture and moderately grazed grazingland with abundance of bushes, as compared to 100% decline (dry) in a colluvial related spring with WNW aspect, agriculture and moderately grazed old-growth chir pine forest land use and a negligible amount of bushes. Springs were also characterized by (i) a peak discharge in rainy season followed by a gradual decline and negligible response to winter rains; and (ii) those who had a sharp decline from peak discharge and substantial response to winter rains. Hydrological characteristics of the springs call for research on 'spring sanctuaries' and adopt better management and technological inputs in order to cater to normal drinking water demand of the catchment people.

WATER as a natural resource and fundamental basis of existence of life is abundant in the Himalaya, but its uneven distribution both in space and time comes in the way of development needs of the region¹. Despite the fact that the mountains provide life-giving water to millions of the downstream people through perennial river system, its people face acute shortage of water during summer. People are sometimes compelled to reduce water consumption, consume unhygienic water, purchase water, and face social conflicts². Another face of the problem is soil erosion, deterioration of agricultural productivity, instability of hillslopes and landslides, and floods and associated catastrophic losses in the adjoining plains caused by the intense rainfall during rainy season^{3,4}. Springs are drying up or becoming seasonal, and the difference in the volume of water flowing down the rivers during dry and rainy seasons is commonly more than 1000 times, resulting in too-little-and-too-much-water syndrome – a common feature of the desert country⁵. Studies^{5,6} indicate that deforestation, land use change, intense grazing, reduced water retention capacity of the catchments, declining rainfall in some localities, etc. have led to diminishing discharge of the springs.

At present, in all hill areas, excluding river valleys, the main sources of sustenance are the sub-surface and

surface water flows which need adequate recharging to meet the demand. The recharging depends upon vegetational cover, in addition to the geological and geomorphological controls in the recharge zones. Geo-hydrological studies suggest that the lineaments produced by joints, fractures, and faults play a very significant role on the hydrogeological regime of a catchment^{5,7}. We still have a limited knowledge about the nature of the springs in response to rainfall, recharge zones, role of vegetation, land use in spring recharge, etc. Equally important are the technological inputs and management issues^{2,4}. This combination of interrelated factors calls for work on 'spring sanctuaries'. The present work provides spring discharge pattern in a mountain watershed, explores interaction of spring behaviour with rainfall, land use, and other morphological characteristics of the springs, emphasizes water resource management and points out the need for research and follow up action.

Located between 30°5'N and 78°46'E and 1650 m asl altitude, Dugar Gad catchment covers about 306 ha area (Figure 1). Annual rainfall (average of 1994 and 1995) amounts to 1694 mm, of which 74% occur during rainy

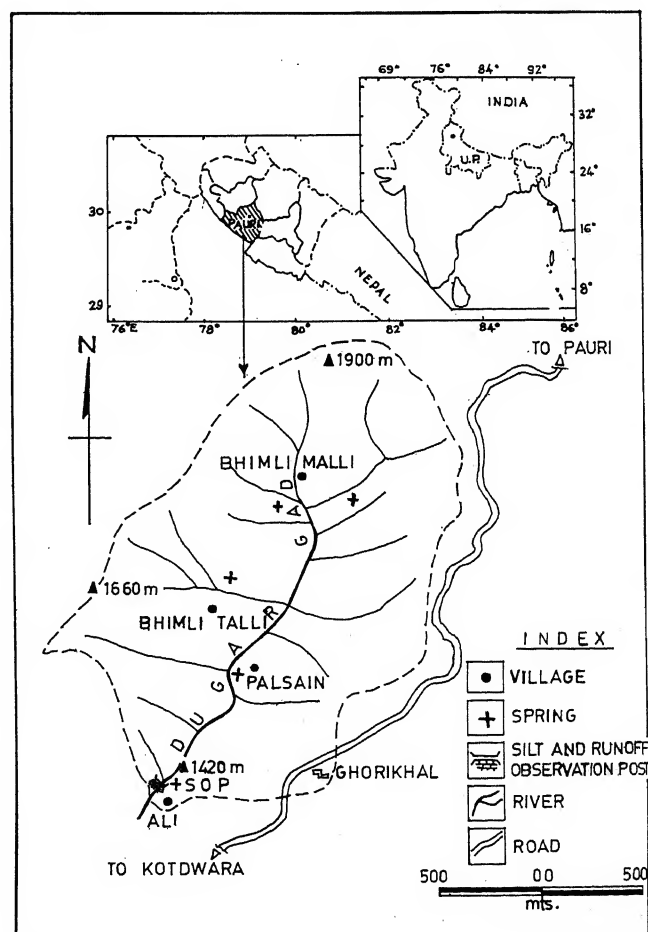


Figure 1. Location of Dugar Gad catchment.

Table 1. Some characteristics of Dugar Gad catchment selected in Garhwal Himalaya. Values in parentheses indicate the seasonal water sources. Data based on ref. 8

Parameters	Dugar Gad
Altitude (m asl)	1400–1900
Land use	
Total area (ha)	306.0
Cultivated area (%)	37.6
Forested area (%)	9.3
Wasteland (%)	53.3
Population	
Human	958
Livestock (au per ha)	6.29
Vegetation	
Fooder/firewood trees	1089
Fruit trees	539
Wild trees (chir pine)	5807
Total number of water sources	
Public tap (functional ?)	21
Spring	16 (10)
Naula	18 (11)
Well	1
Hydrometeorology	
Rainfall ($\text{m}^3 \text{ha}^{-1} \text{yr}^{-1}$)	16940
Run off ($\text{m}^3 \text{ha}^{-1} \text{yr}^{-1}$)	7000.4
Sediment loss ($\text{t ha}^{-1} \text{yr}^{-1}$)	8.62
Average run off efficiency (%)	41.3

season. The mean monthly minimum temperature was 6°C (in January) and the mean monthly maximum was 31°C (in June). Population attributes, land use, water sources and a few other hydrometeorological features of the catchment are given in Table 1. Data of runoff and sediment loss are based on a separate study being undertaken by us in this catchment. Lack of irrigation, low yield, small and scattered holdings (10707 tiny terraces) are some of the reasons which have put about 60% of the cultivated land abandoned in this catchment. Despite a huge number of springs and 'naulas' (very shallow 1–2 m deep, appropriately lined wells to recover water from seepages) discharge in them diminishes during summer which compell people to reduce their household water consumption. Therefore, drinking water provisions and land and water conservation constitute the main challenges in this catchment.

Four springs, one each in the four villages of the catchment were selected for this study in April 1995. In addition, a near-extinct spring (Bhimli Talli-2) was selected for discharge revival employing vegetative and engineering methods. These springs differ from each other with respect to their nature, i.e. perennial (P) and seasonal (S), recharge zone area, upslope aspect, slope, land use, type of rocks and upslope vegetation (Table 2). Geologically these springs were of three types: colluvial related (Palsain; Figure 2a), fracture/joint/

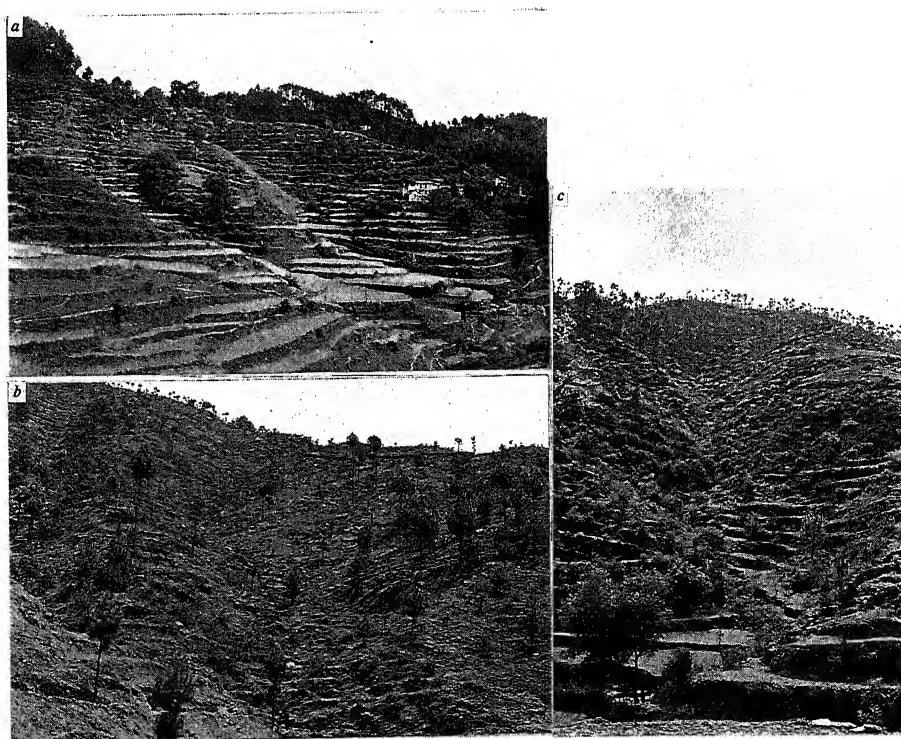


Figure 2. a, Palsain – a colluvial related seasonal spring. The major land use in recharge zone is terraced agriculture and old-growth chir pine forests (in the upper zones). + sign locates the spring. b, Bhimli Talli-1, a fracture/joint/colluvial related perennial spring. The major land use is bush land and grazing land with young chir pine trees scattered in the upper zone. c, Bhimli Talli-2, a fracture/joint related near-extinct spring taken up for discharge revival experiments. The major land use of recharge zone is severely grazed land without trees.

RESEARCH COMMUNICATIONS

Table 2. Location, morphological characteristics, land use and vegetation associated with the springs in the Dugar Gad catchment

Name of the springs (village)	Upslope area (ha)	Type of the spring	Rock types	Average slope of catchment, aspect	Major land use	Vegetation of the spring catchment
Ali (S) (1440)	20.9	Fracture/joint/colluvial related	Phyllite, phyllitic quartzite	36°, SW	Forest under moderate grazing influence	Old growth chir pine (<i>Pinus roxburghii</i>) stand, bushes of <i>Berberis asiatica</i> , <i>Crataegus crenulata</i>
Bhimli Malli (P) (1550)	12.1	Fracture/joint/colluvial related	Phyllitic quartzite	12°, SE	Agriculture and grazingland under low grazing influence	Scattered young trees of <i>Celtis australis</i> , bushes of <i>B. asiatica</i> , <i>C. crenulata</i> and <i>Rubus ellipticus</i>
Bhimli Talli-1 (P) (1500)	40.7	Fracture/joint/colluvial related	Phyllitic quartzite	10°, SE	Abandoned land, shrubland and grazingland under low grazing influence	Young chir pine trees, a few trees of <i>C. australis</i> , bushes of <i>B. asiatica</i> , <i>C. crenulata</i> and <i>R. ellipticus</i>
Bhimli Talli-2 (P) (1575)	18.5	Fracture/joint related	Phyllitic quartzite	40°, SE	Open grazingland under high grazing influence	No trees, bushes of <i>B. asiatica</i> , <i>C. crenulata</i> and <i>Rhus parviflora</i>
Palsain (P) (1450)	10.4	Colluvial related	Phyllite, phyllitic quartzite	15°, WNW	Agriculture and forest under moderate grazing influence	Old growth chir pine forest, scattered trees of <i>C. australis</i>

S, Seasonal; P, Perennial; Values in parentheses indicate altitude (m asl).

Table 3. Cumulative deviation (expressed as per cent from peak discharge) in five springs of Dugar Gad catchment in Garhwal Himalaya

Months (1995-96)	Ali (1355)	Bhimli Malli (1486)	Bhimli Talli-1 (1486)	Bhimli Talli-2 (1486)	Palsain (1355)
April	95.2	36.4	60.7	99.3	91.9
May	100**	37.1	67.3	99.5	96.1
June	100**	42.8	76.1	99.7	100**
July	74.6	16.4	53.5	99.3	83.8
August	11311*	18142*	6.4	171813*	18679*
September	0.6	21.4	12060*	62.2	13.5
October	46.7	36.2	22.9	86.7	56.0
November	66.0	34.4	38.6	96.4	77.0
December	81.4	24.1	42.3	98.1	85.9
January	32.1	27.1	47.4	98.2	79.4
February	24.6	46.4	52.3	96.5	75.1
March	51.5	38.2	55.6	97.9	58.9
Total discharge (l)	1789791	4721883	2448510	7100598	2014501

*Peak discharge (litre per day); **Spring dried up; Values in parentheses indicate total annual rainfall (mm).

colluvial related (Bhimli Talli-1; Figure 2b) and fracture/joint related (Bhimli Talli-2; Figure 2c). Spring discharge was observed once in a week and daily rainfall was recorded at two locations (1460 and 1560 m asl). Discharge pattern was found highly variable across the springs (Figure 3). Bhimli Talli-2 spring recorded both minimum (15.5 l per hour on 13 June) and maxi-

mum (5571 l per h on 19 September) discharge across all the springs, registering a drop of 99.7% from the peak. In this spring of high slope (40°), discharge declined sharply. A low to moderate decline in discharge was recorded in Bhimli Malli (55.6%) and Bhimli Talli-1 (82.9%) springs, respectively. These springs were least affected by rainfall during post-rainy season

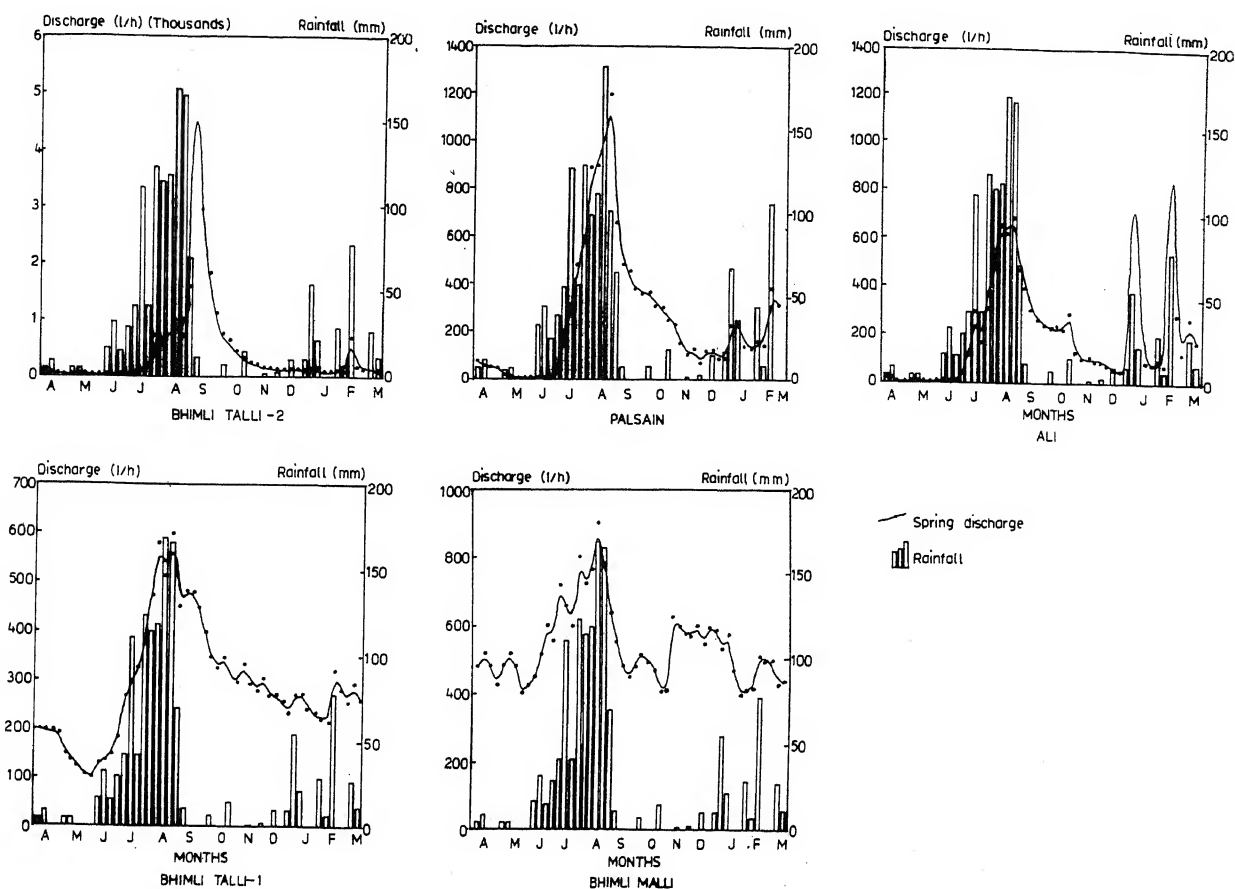


Figure 3. Spring discharge pattern in relation to rainfall in Dugar Gad catchment, Garhwal Himalaya.

(Figure 3). The upslope of these springs is rather gentle, saucer shaped and south-east facing, which allowed less exposure to sun. Recharge zone area and annual water discharge of the springs are unrelated, implying that land use and geological features were equally important. Both of them have a sizeable proportion of abandoned land with thick growth of bushes, low number of mature trees and a low incidence of grazing. Such a situation can be considered ideal for spring recharge zones. Two seasonal springs become dry for 50 days between 23 May and 11 July (Palsain) and for 63 days between 2 May and 4 July (Ali). These springs have old-growth chir pine forests in upper slopes which is under moderate grazing influence. These springs behave in close correspondence to rainfall. It can be emphasized that evapotranspiratory and interception losses, greater slope (Ali), more area under agriculture (Palsain) and year-round grazing; all work together to reduce the moisture retention capacity of the spring recharge areas and render the springs dry during summer.

Among all the springs, Bhimli Talli-2 spring was unique, with respect to land use, high degree of slope, presence of numerous rock outcrops, thin soil layer, ab-

sence of trees and most of the area under year round grazing land. Such landscapes are characterized by a lateral downslope movement of water within the soil layer via a 'quick-flow' process producing typical storm hydrographs⁷. Looking at the hydrological behaviour of these springs, it can be emphasized that each spring has its own character which is influenced by a combination of factors operated in the recharge zone.

Total discharge of the four springs, excluding Bhimli Talli-2 (taken up for discharge revival experiment) in the summer months has the potential to provide 15.6 l water per capita per day to the catchment people, only if the overflowing water is stored in suitable intake structures, distribution system is rationalized and social constraints are undermined. As a matter of fact, Bhimli Malli and Bhimli Talli-1 springs have enough discharge, which often goes waste. On the other hand Ali and Palsain springs are seasonal. People of Ali village located near road head are occasionally provided with water transported from a distant locality by district administration tankers. People of Palsain, a village occupied by the weaker section, have no other option but to dig a well at the point of origin of the dried spring and collect

the dirty water. It can be stated that water shortage is a site-specific problem and needs to be solved combining hydrological and management considerations.

Understanding the site-specific nature of the springs, their response to rainfall, land use, biotic pressure and sociological constraints and limitations of our knowledge with regard to revival of springs, the following areas of problem-solving research in this region may be suggested – (i) Water harvesting, conservation of spring sanctuaries, studying recharge and discharge pattern of springs, collection and analysis of hydrometeorological data (e.g. rainfall, runoff, evapotranspiration, water budget, etc.); (ii) traditional water conservation/management systems; (iii) identification of plants which can help in augmenting ground water recharge; and (iv) strengthening water management system and role of technological inputs to check water misuse and losses.

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Recent crustal adjustments in Dehra Dun valley, western Uttar Pradesh, India

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The compression that was responsible for India-Asia collision and consequent formation of the Himalayan orogen, though subdued, has not yet ceased. A large number of earthquakes occurring between the Main Central Thrust (MCT) and Himalayan Frontal Fault (HFF) bear testimony to the continuing compressional stress regime. The present study provides

evidence of recent crustal adjustments in Dehra Dun valley to the south of Main Boundary Thrust (MBT) in the form of tilted and deformed terraces, colluvial wedges, land subsidence and older rock sequences overriding the Holocene sediments. These neotectonic movements warrant detailed investigations using modern techniques in this seismotectonically sensitive area.

INDIA-Asia convergence that started about late Cretaceous, finally resulted in plate collision in early Cenozoic (45 ± 5 Ma). The deformation front successively propagated towards south. The rate of northward movement of the Indian plate relative to Siberia is estimated to be about 44–61 mm per year¹. Of this, about 10–15 mm per year is absorbed in the frontal Himalayan region while the remainder is distributed across the Higher Himalaya, Tibet, Tein Shan and further north². Some workers^{3,4} visualize MBT as the current boundary of plate convergence. Neotectonic deformation in the foreland basin of Himalaya has been studied in the Nepal sector⁵, Kumaun^{6,7} and Garhwal⁸.

The unusually wide sub Himalayan belt between the rivers Ganga and Yamuna is known as Dun re-entrant area. This area comprises of bedded Upper Siwalik sediments and Holocene Dun gravels and houses the densely populated township of Dehra Dun in the central part (Figure 1). This Dehra Dun valley has been the focus of attention since the 1905 Kangra earthquake that had one of its high intensity zones (secondary epicentre) around this city⁹. Currently this area is rising at the rate of 1 mm per year¹⁰. To map the active faults between the MBT and the HFF, a detailed geological mapping was carried out by the present authors in 1994–1995 (Figure 2).

The foreland basin in the outlined area comprises a basal sandstone succession with interbedded subordinate clays overlain by pebbly sandstone and boulder conglomerate. The boulder conglomerates exposed in the vicinity of the MBT show definite late Upper Siwalik affinity (V. Raiverman and A. C. Nanda, pers. commun.). In the field no tectonic or stratigraphic break was observed between these conglomerates and the underlying sandstone-shale succession. The entire sequence has thus been equated to Upper Siwalik subgroup exposed across Yamuna¹¹. These sedimentary rocks are deformed into a major NW-SE trending syncline, flanked on either side by two parallel trending anticlines. The syncline is largely occupied by Holocene Dun gravels. In the northern range, four fault planes paralleling the MBT have been identified between Yamuna and Rishpana rivers. The fault plane that lies immediately to the south of the MBT follows the axial zone of the northern anticline.

In the southern belt (part of southern anticline), a major zone of back-thrusting is identified between

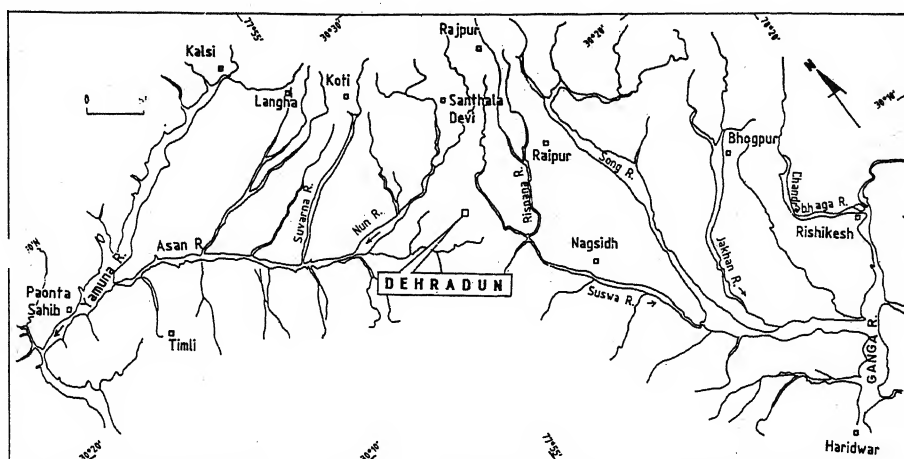


Figure 1. Location map of the area.

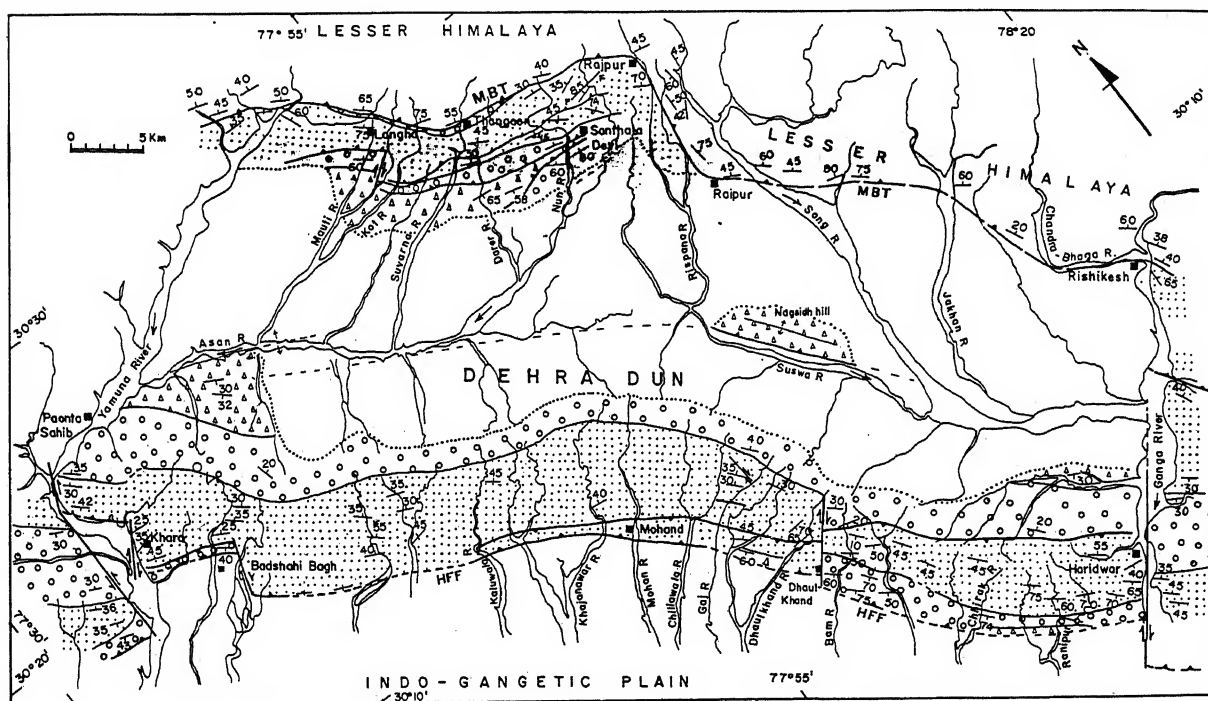


Figure 2. Geological map of the area. Indexed lithologies represent Upper Siwalik Formation. The dotted part indicates sandstone sequence with minor clay, siltstone and pebbly sandstone beds, the open circles indicate dominantly boulder conglomerates with occasional clay, siltstone and pebbly sandstone, the open triangles indicate the youngest Siwaliks deposited unconformably over the Upper Siwaliks and blank space in the central part indicates Holocene Dun gravels.

Kaluwala rao and Ganga. Three major zones of strike slip faulting have been mapped in this belt oriented along the rivers Yamuna, Dhaukhand and Ganga with approximate lateral shifts of about 20 km, 2.5 km and 11 km respectively¹².

A close interrelation between the basement configuration and the structural set up of the area is worked out on the basis of the present studies. In the southern ex-

tremity an E-W vertical fracture is inferred in the basement along which the northern block has subsided. Arrest of the south-directed thrust movement against this vertical fracture initiated back-thrusting in the southern belt. Subsidence along this fracture increases towards east and hence the back-thrusting becomes increasingly pronounced in this direction. The observed back-folds (Figure 3a) overridden by back-thrusts



Figure 3. *a*, Sandstone beds displaying a back fold structure in Khajnawar rao section. Camera views towards west. The southern limb (left hand side) of the fold is overturned; *b*, The back fold is run over by back thrust pushing the thrust slab towards the hinterland. View towards west; *c* and *d*, A north dipping shallow plane cuts across the moderately north dipping boulder conglomerate beds near Rajauli in Kot river section. The pebbles and boulders in the zone are highly fractured. A stretched pebble oriented along the fault plane indicates normal faulting (camera facing west); *e*, Thick column of un-endurated terrace sediments in Darer nala section near Manduwala showing north hading normal fault along which a colluvial wedge has developed (camera facing east).

(Figure 3 *b*) in this zone are the surface expressions of this subsurface fracture. Presence of the vertical fracture in the basement is also supported by the interpretation of the seismic profiles¹³ across the area.

Our study shows evidence of ongoing tectonic activity in the form of tilted terraces, colluvial wedges, faulting in stream terrace, active land subsidence and the older rock sequences riding over the

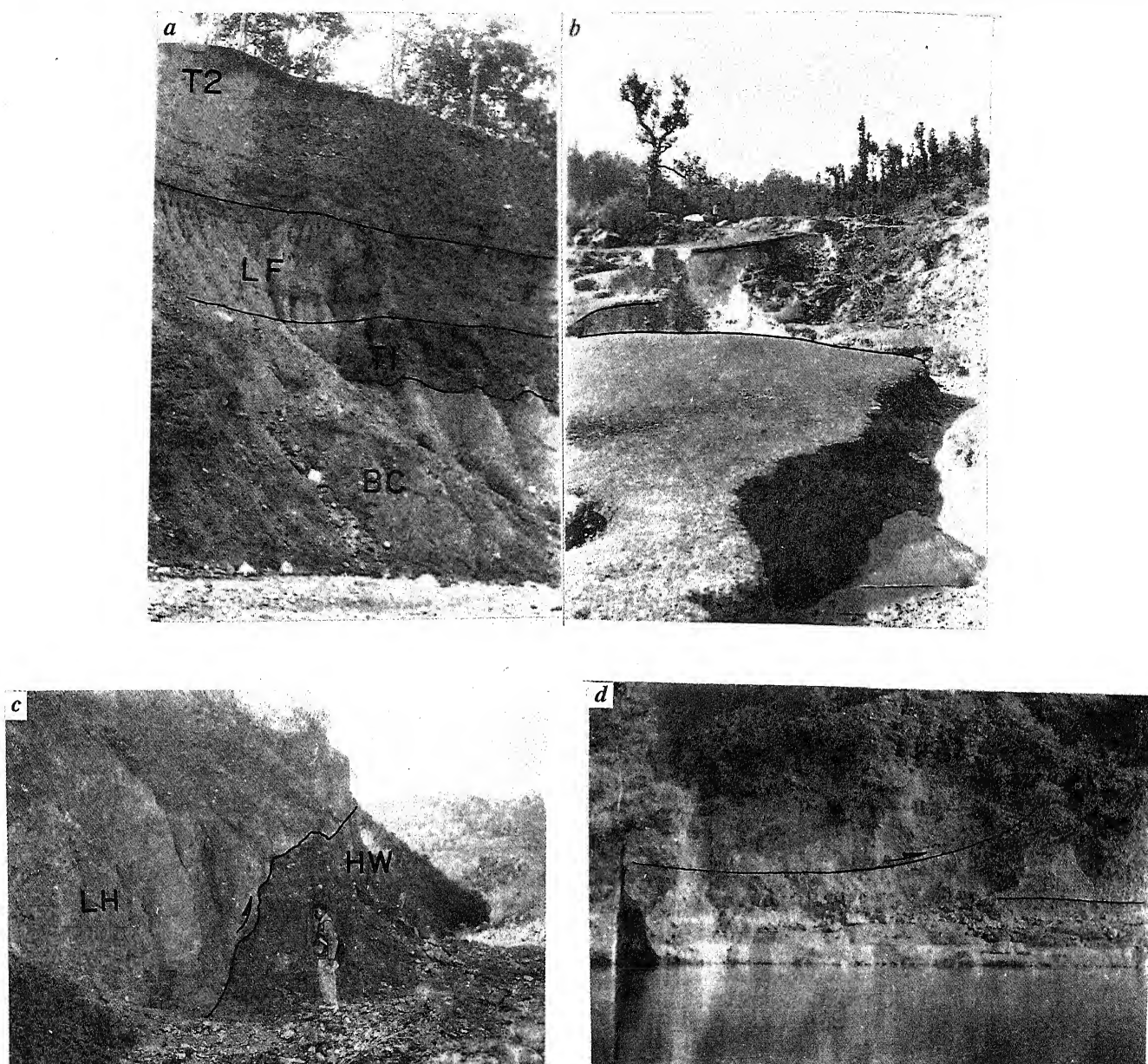


Figure 4. *a*, In the upstream of Darer nala (north of Manduwala) north tilted terrace (T1) is seen resting over the vertically disposed boulder conglomerate formation (BC). The tilted terraces form the base for a nearly 3 m thick fine-grained lake fill deposits (LF), succeeded upwards by unassorted II terrace (T2) of about 5 m in thickness (view towards east); *b*, Displaced terraces near village Khara indicate a zone of subsidence along a recently active fault zone (photograph taken towards west); *c*, East of Rajpur along river Rispana SW directed thrusting along an en-echelon shear plane of MBT (lying in the north) is noticed. The Precambrian Simla Unit (LH) overrides the Holocene hillwash (HW) along this fault plane (photograph taken towards ESE); *d*, SW directed thrusting close to MBT zone in Holocene river terraces near the Chandrabhaga–Ganga confluence in Rishikesh (photograph taken across Ganga on a left bank face).

Holocene sediments. These features are described below.

Tilted terraces are observed in Darer, Kot, Nun and Suvarna river sections. The tilt of the terraces varies between 15 and 25°. A shear zone is recorded in the Upper Siwalik conglomerates in the Kot river section. The conglomerate clasts are shattered and differentially displaced along the shear zones. One of the pebbles was found to be sliced (Figure 3 *c* and *d*). The sense of movement along this NE dipping shear plane is of normal type. The NE trending normal faults in this unit have

been produced due to sudden upliftment of the footwall block and consequent collapse of the hanging wall.

In the Darer river section near Manduwala, a colluvial wedge is observed in a 20 m thick river terrace (Figure 3 *e*), suggesting normal faulting. The fault plane dips 35° towards NE. The terrace deposits are bedded, but poorly assorted and without appreciable breaks. The terraces are tilted towards the north, suggesting uplift in the Quaternary times along a sub-vertical fault parallel to the MBT. This relative uplift of the block caused local ponding in which the fine sediments were deposited

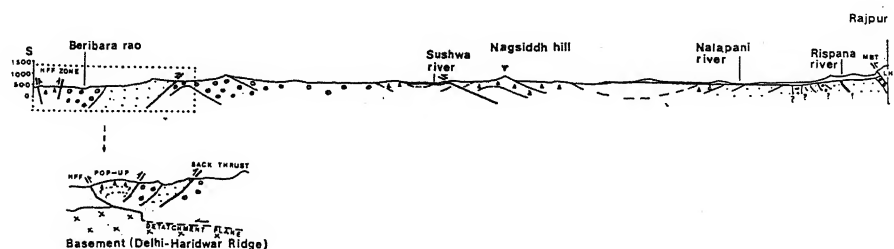


Figure 5. Geological cross-section along Ranipur-Motichur bypass road section in the southern Siwalik belt. The inset shows the pop-up structure in the HFF zone uplifting the Holocene sediments. Geology is as per Figure 2.

in the terraces (Figure 4a). Northward tilted terraces are also observed along stream sections lying to the east of Nun river. A zone of subsidence occurs along a recently active fault to the northwest of Santhaladevi shrine. The land has subsided by about 5 m (Figure 4b).

To the east of Rajpur along the Rispana rao section, an active en-echelon thrust of MBT is identified. Along this thrust, the Lesser Himalayan rocks are overriding the Holocene sediments (Figure 4c). Friction clays and carbonaceous matter are observed along this contact. Similar activities have been noticed near Ganga-Chandrabhaga confluence (on the left bank of river Ganga), Rishikesh where southwest-directed thrusting along a shear plane is observed in the river terraces (Figure 4d).

In the core of Dun syncline an E-W trending thrust has been identified along the southern border of Nagsiddh hill situated to the south of Rajpur. This shallow N-dipping thrust plane brings a folded anticlinal structure in the youngest unit of the Upper Siwaliks (shown by open triangles in the map) over the Holocene gravels and boulders in the synclinal basin. The fault is not exposed towards Yamuna but an anticlinal structure delineated along Asan is indicative of its blind nature. In Dehra Dun city itself, prominent NW-SE (310° - 130°) oriented fractures were observed in a large number of houses in a residential colony across river Rispana. These fractures are continuously opening up. The detailed studies are underway.

Evidence of neotectonic movements is also found along the HFF zone in the southern Siwalik belt between Chhirak rao and Ranipur rao. Here the youngest Upper Siwaliks containing unconsolidated, poorly stratified, boulder-pebble sequence in clay sand matrix display an open NW-SE striking anticline. This structure has formed within a pop-up setting between two thrust faults in the HFF boundary (Figure 5). In a nala section to the east of Bam rao, the displaced pebbles in the conglomerate unit along a SSW dipping shear plane suggest NE directed back-thrusting. Similar structures also occur in the Bam rao section. Neotectonic deformation is also reported in the Dhaukhand strike-slip fault zone along the Gaj rao⁸. Neotectonic adjustment of the rocks here was shown to be responsible for the uplift of the river

bed. From the presented evidence, three distinct neotectonically active domains have been identified: (i) the northern Siwalik belt experiencing active normal faulting, (ii) the central zone with south-directed thrusting and (iii) the southern belt with prominent back thrusting (north directed thrust movement).

Neotectonic movements show active ongoing movement related to the N-S compression responsible for Himalayan mountain building. This compression has not yet ceased and is causing strain in the foreland basin of Himalayas as manifested by the observed structures. Evidence of both SW-directed thrusting, NE-directed back-thrusting together with normal faulting recorded in the area suggests that the deformation is dominantly being controlled by the basement topography. The presence of active thrusting along an en-echelon shear of MBT and a thrust plane on the southern margin of the Nagsiddh hill in the eastern part of the area indicate that movement along HFF has almost ceased and currently some part of the strain is being released along these newly-formed shear surfaces.

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The plague outbreaks of India, 1994 – A prologue

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PLAGUE is a disease of great antiquity in India. India was one of the countries most severely affected by the Third Pandemic of plague which spread around the world through steamships; there were an estimated 12.5 million deaths due to plague between 1886 and 1950 in India¹. In his autobiography *My World of Preventive Medicine*, C. G. Pandit recollects the night sky of Bombay lit up every night by the rising flames of burning bodies, dead from plague in the early part of this century². From 1950 onwards with the increasing availability of DDT and broad-spectrum antibiotics, plague transmission was gradually curtailed and mortality from the disease much reduced. India's Haffkine Institute played an important role in the history of plague in developing an anti-plague vaccine as early as the turn of the century followed later by treatment trials of patients affected with plague. By 1967, interruption of wild plague transmission to humans appeared to have been achieved in India and human plague outbreaks were no longer reported. Between 1967 and 1993 there were reports of two separate outbreaks of plague but neither of them was confirmed as plague³. It is well known that plague cannot be eradicated just as small-pox was and poliomyelitis would hopefully be in the near future. It is a disease of the earth, of the creatures that run and burrow the earth and of the fleas. Man is an accidental victim and need not have plague infection for plague persistence. In recent decades, plague had simply retreated to rural natural foci of infection, involving mostly wild rodents and their fleas with occasional spillovers to commensal hosts and humans in villages and towns¹. Many natural foci of plague in wild rodents exist today not only in India but throughout the developing world in the continents of Asia, Africa and Latin America. Meanwhile, there had been a shift in plague mortality and morbidity from its dominance in Asia in the 1960s and 1970s to Africa in the 1980s and 1990s with case fatality rates still remaining high.

But epidemics of human plague can be prevented from occurring and controlled with present-day knowledge and methods of surveillance, prevention and treatment. This is what did not happen in India, repeating the usual story of complacency following initial victory against communicable disease⁴. Unstable epizootic factors with

hostless fleas are conducive to the outbreaks of human plague.

Thus it was that between August and October, 1994 India was struck by two outbreaks of plague in succession – one of suspected bubonic plague in the Beed District of the State of Maharashtra and the other of suspected pneumonic plague in the Surat city of the State of Gujarat. On the basis of circumstantial and serological evidence the outbreaks were presumed to be plague and they were rapidly brought under control by governmental action at the State level assisted by the Centre. In the process, however, India's vulnerability to emerging and re-emerging infectious diseases was exposed on many counts, especially in the areas of epidemiological monitoring and supportive laboratory services, the essential pre-requisites for rapid response. As the WHO International Team investigating the plague outbreaks in the country said in its Executive Report dated 22 November, 1994, while the outbreaks were raging, there were problems with the investigating laboratories in being able to quickly differentiate real cases of plague from the huge background load of a variety of infectious diseases which also cause epidemics with similar clinical and epidemiological features⁵. 'Diagnosis became overly dependent on procedures that are used to support a diagnosis of suspected plague (Gram stain, Wayson stain of smears prepared from clinical materials). A similar over-reliance was placed on serologic procedures that support a diagnosis of presumptive (probable) plague. (Single serum specimen testing using passive haemagglutination assay)'⁵. May C. Chu, the microbiologist member of the WHO International Team, who comes from the WHO Reference Laboratory for Plague at the CDC in Fort Collins, Colorado, USA, said on November 18, 1994: 'Though there were multiple attempts to isolate *Yersinia pestis* from sputum, blood and autopsy specimens, there is no clearly-identified *Y. pestis* culture associated with any specimen obtained from suspected plague patients.'⁶ Thus in those early days, diagnostic uncertainty and confusion prevailed; alternative diagnoses implicating other infectious agents were offered and there was panic and excessive alarm on the part of the public, coupled with widespread reporting by the media. The message

did not go through effectively that in the modern era with its array of insecticides, diagnostic and therapeutic tools, today's plague is not the Black Death of olden times and is readily amenable to prevention and control through a system of surveillance and prompt response. Fifteen airlines cancelled 400 flights to India, resulting in much economic damage.

Against this background, on 9 October, 1994, by which time the two outbreaks had largely subsided, the Government of India constituted the Technical Advisory Committee (TAC)* on Plague with the following Terms of Reference:

1. To elucidate factors responsible for the current outbreak of plague and its spread;
2. To advise on strategies, policies and programmes for the control of plague;
3. To recommend steps for prevention of such outbreaks in future.

TAC initiated work on all the three Terms of Reference but even at the outset it was clear that the on-going controversy in the media and in professional circles as to whether it was plague or not would not abate until conclusive evidence of the identity of the causative agent was found. And unless this was done, TAC could not proceed with addressing its Terms of Reference.

There was, however, a major snag. The outbreaks had largely subsided by the time TAC was formed and so it was not possible for TAC to collect and study fresh material for laboratory confirmation of human plague through isolation of *Y. pestis* from clinical specimens. TAC had to have recourse to the original clinical specimens obtained while the outbreaks were 'on' and stored in Surat and at the National Institute of Communicable Diseases (NICD) in Delhi. The WHO International Team had remarked that attempts at culture isolation and identification of *Y. pestis* at both these places had failed to yield pure characterizable colonies⁶. Contamination of bacterial isolates was a problem. TAC then decided to mobilize the expertise and laboratory facilities available within the country, including those with some members of TAC for this task. Both the new Civil Hospital in Surat and NICD, Delhi, had extended their full cooperation to TAC in providing the stored cultures

for TAC's study. The Defence Research and Development Establishment (DRDE) in Gwalior (H. V. Batra) had undertaken the main task of isolation and characterization of *Y. pestis* from the stored cultures and further molecular characterization of the isolates in a pure form was carried out by scientists working in a network of laboratories functioning under various research agencies in India. Scientists in the following institutions played a key role in this effort: the All India Institute of Medical Sciences (AIIMS) (S. K. Panda); the Post-Graduate Institute of Medical Education and Research (PGIMER), Chandigarh (N. K. Ganguly); the Institute of Microbial Technology (IMTECH), Chandigarh (Amit Ghosh); and the Centre for Cellular and Molecular Biology (CCMB), Hyderabad (S. Shivaji). Meetings of the scientists from these institutions were held from time to time and there was a spirit of cooperation and team work amongst them in this endeavour. H. Sharat Chandra of the Indian Institute of Science, Bangalore and a member of TAC coordinated this work. In addition to clinical material from Surat, specimens of original rodent material and of fleas from Surat and Beed collected by NICD scientists during and after the outbreaks were made available to TAC for culture isolation. In the entire work done by TAC and the expert panel to track down the infectious agent, the Health Departments of the State Governments of Maharashtra and Gujarat gave their utmost cooperation.

The result was that *Y. pestis* could be isolated and characterized as such from some of the clinical samples in Surat and from the environmental samples in Beed and Surat. In this issue of *Current Science*, a number of articles appear, narrating the story of isolation and characterization of *Y. pestis* and providing TAC's response to the Terms of Reference. At first there is an overview of the work done by TAC followed by a series of articles on the isolation and characterization of *Y. pestis* from the clinical and environmental samples, the serological evidence, a special article on molecular characterization of *Y. pestis* isolated from the outbreaks, followed by ecological aspects of the outbreaks. These articles are written by scientists who had carried out the work in their laboratories. In the end, the epilogue looks at the future scenario – what next?

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An overview of the work carried out by the Technical Advisory Committee on plague

DURING August, 1994 the village Mamla of Beed district of Maharashtra State experienced unusually heavy flea nuisance and ratfalls. Subsequently, a number of cases clinically resembling bubonic plague were reported from Mamla and nearby villages of Beed district. Making a presumptive diagnosis of bubonic plague outbreak and based on the totality of ecological, clinical and serological evidence, the health machinery of the State Government with the support of the Central Government took prompt containment measures.

During September, 1994, government hospitals and private clinics in the City of Surat in the neighbouring State of Gujarat reported an increasing number of patients with a highly fatal illness resembling acute pneumonia. Based on clinical, laboratory and radiological findings a presumptive diagnosis of an outbreak of pneumonic plague was made. The State Government, again with the active support of the Central Government, rapidly instituted containment measures.

The establishment of the Technical Advisory Committee (TAC) on plague, its terms of reference and pattern of working are described in the prologue.

It was plague after all – the evidence

The organism

DRDE, Gwalior had succeeded in obtaining pure cultures of *Y. pestis* in 11 out of 82 samples processed by them from patients suspected of pneumonic plague from Surat. They also succeeded in obtaining 6 *Y. pestis* isolates from different batches of trapped rodents from Beed within a year of the outbreak and another single rodent isolate from Surat City from among 17 animals in August, 1995. Confirmation and characterization of *Y. pestis* was done according to standard procedures such as gross characteristics, colony morphology, staining, biochemical tests, fluorescent antibody to F1 antigen, specific bacteriophage lysis, counter-immuno-electrophoresis and SDS-PAGE analysis. H. V. Batra *et al.* describe (page 787) this work in the paper 'Isolation and identification of *Yersinia pestis* responsible for the recent plague outbreaks in India'.

Confirmation that a case or an outbreak is one of plague rests on cultural isolation of *Y. pestis* from clinical specimens obtained from patients with clinical and epidemiological features compatible with plague. The

isolates should show lysis by specific bacteriophage and a biochemical profile indicative of *Y. pestis*. This has now been accomplished. The long gap in processing specimens from the time of their collection and attempted culture with consequent deterioration, and the heavy overgrowth of contaminating organisms made culture isolation a difficult process and may explain the low yield of positive cultures for *Y. pestis* (11 positive out of 82 pneumonic patients of Surat city). It is of interest that 9 out of 11 positive cultures were from patients in the early days of the outbreak, presumably before widespread antibiotic use in the community.

Plague can also be confirmed through the passive haemagglutination (PHA) test by demonstrating a four-fold difference in serum antibody titre between specimens taken at appropriate intervals (minimum of 2 weeks) and by demonstrating their specificity for the F1 antigen of *Y. pestis* as determined by haemagglutination inhibition (HI). The serological investigations in Beed and Surat outbreaks were handicapped by lack of availability of paired serum samples at appropriate intervals. Acute phase sera were generally not available. Serological evidence of plague in this study rests largely on high antibody titres in a majority of the serum samples (85.2%) taken from patients in Surat convalescing 2 to 3 months following illness. It is important to note, however, that in all cases, the specificity of passive haemagglutination test was ensured by simultaneously performing a haemagglutination inhibition (HI) test. These results, taken together with specific sero-reactivity to the F1 antigen in humans and dogs in Beed and Surat, reported by the WHO International team provide further confirmatory evidence that the outbreaks in Beed and Surat were due to *Y. pestis* infection. The serological work is presented in this series in the paper entitled 'Passive haemagglutination tests for *Y. pestis* infection in Surat pneumonic patients' by G. S. Agarwal and H. V. Batra (page 792).

Tissue pathology

Small tissue samples made available to the TAC from 7 autopsies from the New Civil Hospital were examined histopathologically. While showing no consistent pattern of pathology, there was a variable picture consisting of some cases showing intra-alveolar oedema, haemorrhage and a pneumonic process in the lungs of varying sever-

ity. *Y. pestis* could not be demonstrated in these tissue sections.

Molecular characterization of *Y. pestis* isolates

Molecular characterization of bacterial isolates from pneumonic plague patients in Surat and from rodents and fleas in Beed and Surat together with PCR analysis of tissues obtained at autopsy of 7 patients who died of pneumonic plague, provided a valuable dimension to the study of the Indian plague outbreaks of 1994. These aspects are discussed (page 794) in the paper entitled 'The 1994 plague epidemic of India: Molecular diagnosis and characterization of *Yersinia pestis* isolates from Surat and Beed' by S. K. Panda and coworkers in this series.

PCR analysis of autopsy tissues from the 7 patients who died of pneumonic plague, using primers for *Y. pestis*-specific *pla* and *f1* genes revealed that 5 were positive for *f1* gene and 2 for the *pla* gene. The results provided unequivocal evidence of tissue invasion by *Y. pestis* and a further confirmation of the Surat outbreak being plague. This is perhaps the first time that *in situ* demonstration of *Y. pestis* in historical material has been attempted successfully.

All the 18 bacterial isolates (11 from human cases in Surat and 7 from rodents in Beed and Surat) were positive for the presence of *pla* and *f1* genes by PCR.

Ribotyping has proved to be an extremely useful tool for molecular typing of *Y. pestis* isolates. Upon ribotyping, the Indian isolates were identical to one another, whether derived from patients with pneumonic plague or from rodents of Beed and Surat but differed from all the *Y. pestis* strains examined so far by Elizabeth Carniel of the Pasteur Institute, Paris at the WHO Reference Centre on *Yersinia*. Although different from the 16 previously known ribotypes, it does not follow that the Surat strain is new and of recent origin since only 85 of the approximately 6000 *Y. pestis* isolates known to exist in *Y. pestis* collections worldwide have so far been ribotyped. It is noteworthy that Surat and Beed seem to harbour *Y. pestis* of the same ribotype.

Pulsed field gel electrophoresis confirmed that all the three plasmids of *Y. pestis* of molecular weights 7.5, 75 and 110 kb were present in the Surat isolates. This implies the presence of virulence-associated genes and also the conservation of F1 antigen as demonstrated by immunoblotting, giving confidence to the F1-based serology for diagnostic and epidemiological work.

With regard to the presence of an extra protein band in the 25 kd region of Surat strains, initially observed in joint studies carried out at the WHO International Reference Centre on Plague at Fort Collins, Colorado, USA, it is not clear what significance should be attached to this protein band.

What does all this mean? The molecular identity of *Y. pestis* isolates from human cases of pneumonic plague in Surat and of 6 *Y. pestis* isolates from different batches of trapped rodents from Beed within a year of the outbreak and a single rodent isolate from Surat suggest that that isolates were clonal in origin; the possibility is also raised of an epidemiological linkage among them. The organisms that were obtained from both locations might have been derived from pre-existing ones in each of the areas. The possibility that the organisms might have travelled through the movement of rodents and humans cannot be ruled out, given the fact that frequent movement of rodents and their household effects are known to take place between Beed and Surat.

The molecular identity of the isolates from human cases in Surat and of the isolates from environmental samples (rodents) from Beed and Surat plus the fact that 5 out of 7 flea samples collected during the outbreak period were positive for the *f1* gene of *Y. pestis* suggest that plague was endemic in natural foci in these areas, possibly generating human outbreaks consequent upon major changes in human ecology. Two papers presented in this series entitled 'Ecology of flea-transmitted zoonotic infection in village Mamla, District Beed' (page 800) and 'Observations on urban ecology of Surat and bubonic plague transmission in the city' (page 803) by V. K. Saxena and T. Verghese throw interesting light on this problem. All in all, TAC was of the opinion that the evidences obtained so far do not indicate that the isolates of *Y. pestis* from the plague outbreaks in India 1994 were the product of genetic manipulation and introduction from outside, a popular theory in the media at that time. Admittedly, there are many gaps in our knowledge of the Indian plague outbreaks of 1994 – bubonic plague in Beed and pneumonic plague in Surat and what connection there may be between the two – which only a continued and active programme of research can fill.

Epidemiological and ecological aspects

As far as the eco-epidemiological background to the outbreak of suspected bubonic plague in the Beed district of Maharashtra is concerned, village Mamla was the index village and the cascade of evidence is as follows, as constructed by the Directorate of Health Services of Maharashtra state:

September, 1993: Earthquake in the neighbouring district of Latur.

September 1993 to July, 1994: Abandoned homes, stored food grains left behind, exponential growth of rat population.

5 August, 1994 onwards: Unusual flea nuisance in Mamla, followed by rat falls in the locality.

26 August, 1994 onwards: Cases with fever and lymphadenopathy started to appear and were reported at the

nearest Primary Health Centre in Kuppa in the area, mass antibiotic prophylaxis and insecticide spraying started on 29 August.

8 September, 1994 onwards: Seropositivity for plague first reported, rising in frequency later, containment measures continued.

20 October, 1994: No new cases reported.

25 October, 1994: WHO declared that the outbreak had been contained.

With regard to the Surat outbreak, during the first week of September, 1994, the monsoon was unusually heavy, a record in the past 25 years; flood waters entered the city and remained stagnant for 5 days; a large number of dead animals were found after the floods receded. Then came the Ganapati festival with close intermingling of huge crowds of people. On 19 September, 1994, an illness characterized by fever, cough, haemophysis, dyspnoea and pulmonary infiltrates on X-ray, in young adults, not responding to penicillin and attended by high initial mortality broke out in Surat city. A suspected clinical diagnosis of pneumonic plague was made by the physicians at the New Civil Hospital, Surat. The patients responded well to tetracycline, aminoglycosides and chloramphenicol. Case fatality rates came down rapidly after the high levels in the first few days. Blood specimens obtained from patients with suspected pneumonic plague revealed sero-positivity to the F1 antigen of *Y. pestis* in 146 out of 1027 samples in a study by the NICD. Reference had already been made to the study by DRDE, Gwalior, wherein 85.2% of serum samples from patients convalescing from what clinically looked like pneumonic plague were positive for antibodies to F1 antigen. Some evidence exists of a clustering of cases and death; taking of antibiotics had a beneficial effect in preventing deaths. The State Health and Civic authorities responded rapidly by instituting active case detection and treatment, providing accurate information to physicians and public on early detection, case management, antibiotic prophylaxis and treatment. 500,000 persons left Surat city in panic to other places in Gujarat, to Bombay and to various destinations in the country, but in none of the urban centres of Bombay, Calcutta and Madras was there any confirmation of plague transmission. The one patient in Delhi who had an enlarged painful lymph node in the right groin with a four-fold rise in titre after haemagglutination inhibition actually travelled to Delhi from rural Maharashtra. No imported plague was reported in any other country.

The lessons learnt

Surveillance of plague is weak in India and needs to be considerably strengthened and expanded. Prompt action to identify the causative agent in a suspected outbreak is of the utmost importance and capabilities for identifica-

tion of plague bacillus as the aetiological agent need strengthening. State Public Health Laboratories are in need of considerable upgrading of skills to make prompt and accurate diagnosis of plague and other infectious disease agents. Paradoxically, while there is expertise in the country for the study of microbes at sub-cellular and molecular levels, diagnostic capability for the isolation and characterization of common infectious agents at the peripheral levels of the health care system is weak. As a result, a vulnerability in India's health care system relating to rapid diagnosis of infectious disease outbreaks exists. There is little preparedness in the provision of Quality Laboratory Services for accurate confirmation of clinical diagnosis. Current plague experiences revealed that rapid mobilization of institutions and experts around the country in time could have helped in the prompt identification of the causative organism. Proper interaction with the media and the public and seeking their assistance are of critical importance. With the information technology available today, much of the surprise and panic among the national and international communities could have been avoided.

Future prevention and control of plague

Surveillance and response should be regarded as the foundation of infectious disease control, be it plague or any other. India has the potential of being well-served in this regard because of the extensive health infrastructure built over the past few decades. This structure is, however, somewhat handicapped by inadequate interaction between the various levels of care and the network of laboratories existent in the country. Prompt backup support of laboratory services of quality in determining the aetiological agent expeditiously must be ensured in future strategies. A national surveillance and response system in India for the control and prevention of infectious diseases is an urgent necessity. TAC has recommended the following steps:

1. Organize a national debate on the strengths and weaknesses of the existing surveillance system and draw up priorities and build on existing strengths.
2. This should lead to the formation of a National Apex Committee on Surveillance and Response for the Control of Plague consisting of appropriate experts from different disciplines and government and non-government sectors. The committee should be headed by an epidemiologist of eminence.
3. Epidemic preparedness should be integral to the control strategy.
4. The formation of a multi-disciplinary action group would be valuable. The group may consist of an epidemiologist, a clinician, a microbiologist and relevant public health and civil authorities. The group will provide guidelines for case definition,

case management, preventive action and information and communication. The National Reference Centre for plague, which in this case, is the NICD, needs to be strengthened in its microbiological and molecular genetic capabilities. There could be different national reference centres for different diseases depending upon expertise availability.

5. A network of laboratories throughout the country must be identified for providing support to the diagnosis of infectious diseases. The surveillance must include surveillance of emerging drug resistance of plague and other pathogens.
6. NICD and the network should be involved in a continuous process of training and re-training of professionals and technicians in the investigation of plague outbreaks and in establishing aetiology.
7. The capacity for continuous surveillance of animals, vectors and human populations for plague within the overall infectious disease surveillance system has to be developed and maintained. The

state and district health services should be the focal points of this system with assistance from the NICD and the national laboratory network.

8. The capacity to respond to plague outbreaks in humans and plague epizootics must be ensured through the development of epidemiologic, ecologic and laboratory investigative capacity.
9. An automated reporting system extending from health centres and laboratories to public health action programmes would be of value.
10. The TAC makes a strong plea for building a well-trained cadre of epidemiologists in the country and in this, medical colleges, schools of public health (where are they?) and specialized institutions devoted to the study of infectious diseases under the ICMR and the NICD will have to play an important role.
11. The value of continuous interaction of the national networks with international networks under WHO and others is inestimable and must be maintained.



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REGISTRAR

Isolation and identification of *Yersinia pestis* responsible for the recent plague outbreaks in India

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The district of Beed in Maharashtra state, India experienced a suspected bubonic plague outbreak in August, 1994. A month afterwards, Surat city, in Gujarat state was in the grip of an epidemic with a highly fatal illness resembling pneumonia. The present work describes the isolation and identification of *Yersinia pestis*, the causative agent of plague, from these outbreak areas. Eleven *Y. pestis* isolates were recovered from 82 pneumonic patients of Surat city. From Beed district, 6 *Y. pestis* isolates could be obtained from different batches of trapped rodents within a year of the outbreak. A single rodent isolate from Surat city was obtained from among 17 animals in August, 1995. Characterization of the *Y. pestis* isolates was done by biochemical tests, specific bacteriophage lysis, counter immunoelectrophoresis, fluorescent antibody test and SDS-PAGE analysis.

PLAGUE epidemics and pandemics have had devastating effects on human beings throughout the recorded history. It was a highly dreaded and fatal disease in the pre-antibiotic era and at times wiped out nearly half the human population in the affected countries. Worldwide, in the post-antibiotic period, plague has existed mainly as a sporadic disease with occasional epidemic outbreaks. According to WHO reports, between 1980 and 1986, many countries in Africa, Asia and America covering a substantial proportion of the globe had reported plague cases¹. During the last decade, outbreaks of both bubonic and pneumonic forms have occurred in Myanmar, Vietnam, Tanzania, Zaire, Peru and Madagascar².

In India, plague along with cholera and small pox formed the 'trinity' of major epidemic infections until the 19th century and thereafter plague outbreaks continued up to 1968 with final disappearance in 1969. Though suspected outbreaks were reported in Maharashtra in 1975 and in Himachal Pradesh in 1984 (ref. 3), but conclusive evidence was lacking. Thus, after the disease was thought to have been conquered, suspected cases of bubonic plague were reported in village Mamla, District Beed of Maharashtra in August, 1994 following typical sequences of flea nuisance and rat falls. Within a

month the industrial city of Surat in the neighbouring state of Gujarat reported an increasing number of patients with a highly fatal form of acute pneumonia. During the period 21 September – 20 October, 1994 a total of 1027 suspected cases were admitted to hospitals in Surat city, of which 146 were recorded as presumptive pneumonic plague based on seropositivity.

We report here the confirmatory evidence of *Yersinia pestis* isolation from human pneumonic patients of Surat city and from the rodents of both the outbreak areas.

Materials and methods

Human samples

Since during the suspected bubonic plague outbreak in district Beed no bubo aspirates or blood samples were available, *Y. pestis* isolation could not be attempted from human cases. From among the patients in Surat presenting a clinical picture of acute pneumonia with fever, cough, haemoptysis, dyspnoea affecting mainly the young adults, 82 sputum specimens could be processed for isolation work. Samples were collected by the Department of Microbiology, Surat Medical College, Surat.

Rodent samples

Tissue samples of spleen and liver from 30 rats (*Rattus rattus*) of Mamla village, district Beed and 4 rats (*Rattus rattus*) from Surat city were collected during August to November 1994. During the months of April and May, 1995, 11 rats (*Rattus rattus*) and in July and August, 53 rats (*Rattus rattus*) trapped from Mamla village and the adjoining Chinchoti village of district Beed were processed. At the same time 17 rodents belonging to *Tatera indica* trapped from the field areas around these villages were also processed for isolation work. Spleen and liver of these animals were kept in Cary Blair transport medium and processed in the laboratory. From the Surat city in the month of July and August, 1995, 17 *Rattus rattus* trapped from the household vicinity of the affected

four localities were also processed. Majority of rodent samples from outbreak areas were received through the dedicated efforts of Shyamal Biswas and his team of Plague Surveillance Unit, NICD, Bangalore.

Isolation procedures

The samples were either streaked directly or after 1 to 2 days incubation in peptone water/brain heart infusion broth at 28°C onto brain heart infusion agar (BHIA), desoxycholate citrate agar and blood agar (BA). Media used were from Diffco Laboratories. The agar plates were incubated at 28°C and 37°C for 24 to 48 h. All the samples yielded mixed growth of organisms. The suspected colonies were purified by repeated sub-cultures and tested for oxidase reaction, Gram staining and Wayson staining. The oxidase negative, Gram negative and Wayson bipolar safety pin positive colonies were processed for biochemical reactions. The biochemical tests performed were indole production; citrate utilization; hydrogen sulphide production; urea hydrolysis; fermentation of glucose, lactose, mannitol, sucrose, maltose, arabinose, xylose, rhamnose, adonitol, dulcitol, cellobiose and salicin; decarboxylation of lysine and ornithine; hydrolysis of arginine.

Bacteriophage lysis

Organisms exhibiting biochemical reactions characteristic of *Y. pestis* were subjected to bacteriophage lysis test. *Y. pestis* monospecific bacteriophage kindly supplied by May C. Chu (WHO Collaborating Center, CDC Laboratory, Fort Collins, USA) was utilized for the test. Phage lysis test was performed using bacteriophage reagent on streaked pure cultures on blood agar plates and incubated at 28°C. *Y. pseudotuberculosis* strain 1 A and *Y. pestis* strain A1122 kindly provided by May C. Chu through Technical Advisory Committee on Plague, Govt. of India, served as controls.

Fluorescent antibody test

A loopful of heat killed overnight broth cultures of four *Y. pestis* isolates, *Y. pseudotuberculosis* strain 1 A and *Y. pestis* strain A1122 fixed with precooled acetone were tested following standard protocol using rabbit anti F1 FITC conjugate received through the courtesy of WHO Reference Centre using Nikon Microphot Universal microscope.

Counter immunoelectrophoresis

Wells were punched off at a distance of 5 mm in agarose (0.8% in veronal buffer, pH 8.6) coated on grease-free

clean glass slides. Wells, towards cathode were filled with crude antigens of *Y. pestis* isolates and other bacteria (*Y. pseudotuberculosis*, *Y. enterocolitica*, *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhi*) prepared by sonication (5 watts, 20 cycles of 60 seconds each using Misonix ultrasonicator) of harvested growth from solid media. Hyper immune sera raised in rabbits against purified F1 antigen of *Y. pestis* was added in the well towards anode. Counter immunoelectrophoresis (CIE) was carried out in an anodic current of 20 mA for 45 min using 0.075 M veronal buffer, pH 8.6 as per the method of Grabar and Williams⁴. After this the slides were incubated at room temperature overnight and washed with normal saline before staining with Coomassie brilliant blue.

Polyacrylamide gel electrophoresis

Bacterial cells were grown on blood agar overnight at 28°C from a single colony. The sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was run as per the method of Laemmli⁵. Briefly, the cells were pelleted and washed in 0.5 M phosphate buffer saline. The pellets were resuspended in 50 µl distilled water and 50 µl SDS-PAGE loading dye. The samples were boiled for 4 to 5 minutes and run in a 10% acrylamide gel at 25 mA till the dye front was close to the bottom of the gel using electrophoresis unit of Atto Corporation. The protein bands were visualized by staining with Coomassie brilliant blue as per the standard protocol.

Results

The colony characteristics of suspected *Y. pestis* isolates on BA and BHIA were not clear following 24 h incubation as the colonies were barely visible. At 48 h, the size of the colonies increased to 0.5 mm in diameter and upon further incubation the size could reach up to 1–2 mm. The colonies on BA were greyish, non-mucoid, round and dome-shaped with a tendency of flattened irregular border on prolonged incubation. At times the appearance of the colonies typically resembled fried egg shapes (Figure 1).

The microscopic appearance of the organisms was coccobacilli to rods with rounded ends and slightly convex sides. Typical bipolar safety pin shapes were visible from young cultures in broth medium following Wayson staining (Figure 2). Organisms in the broth did not produce turbidity, instead floccular deposits at the bottom and rising along the walls of the tubes were very consistent and characteristic (Figure 3).

The suspected *Y. pestis* isolates from human and rodent samples showed positive biochemical reactions to

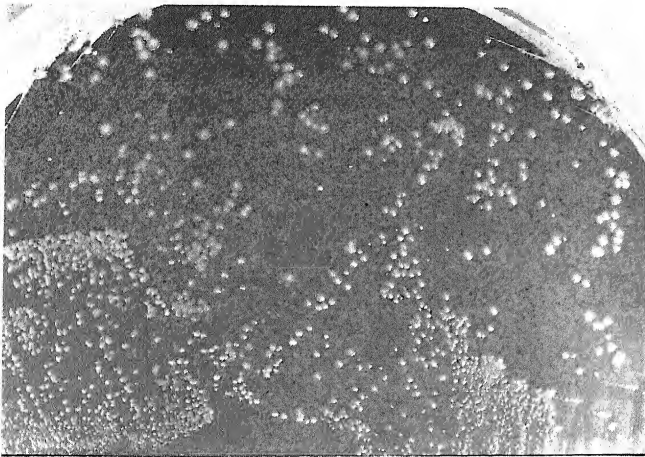


Figure 1. Colonies of a clinical *Y. pestis* isolate on blood agar plate following 72 h incubation at 28°C.

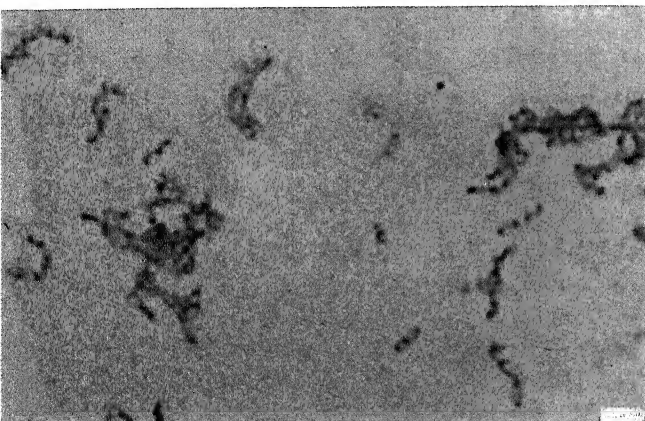


Figure 2. Wayson staining of a clinical isolate of *Y. pestis*.

glucose, mannitol, maltose, arabinose, xylose, salicin and scored negative in the remaining tests.

A total of 22 isolates exhibited these biochemical reactions. However, only 18 isolates could be lysed by the specific bacteriophage. SDS-PAGE revealed a characteristic uniform protein profile for all the 18 bacteriophage positive isolates (Figure 4 *a, b*); the other four biochemically resembling isolates had a very different pattern. Though the pattern of protein bands of the 18 isolates was nearly identical, a slight variation in the region of 34–35 kDa was observed for isolate numbers 9, 10 and 12, wherein a doublet could be seen that was not there in the rest of the samples.

Of the 18 *Y. pestis* isolates described above, 11 were recovered from the pneumonic patients and 7 from rodent specimens (Table 1). The age and sex of the patients yielding *Y. pestis* isolates are given in Table 2.

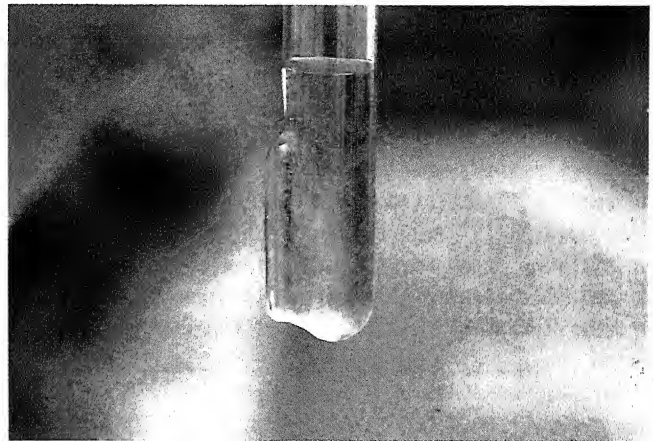


Figure 3. Growth of *Y. pestis* in broth tube.

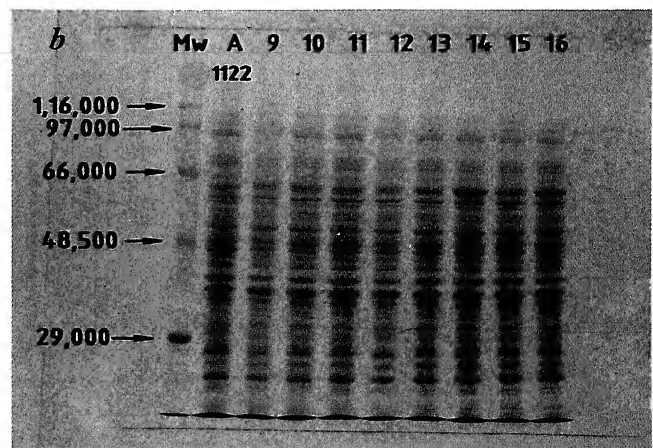
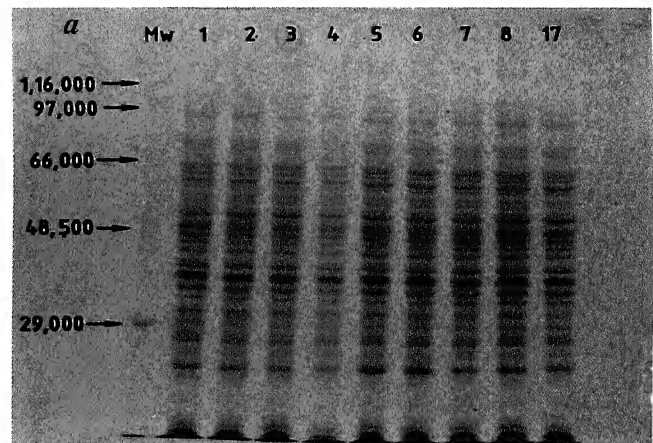


Figure 4 *a, b*. Protein profiles of *Y. pestis* isolates using 10% SDS-PAGE.

Due to the limited amount of anti F1-FITC conjugate available, only 4 of the *Y. pestis* isolates (nos. 1, 2, 3 and 4) could be tested. All the four showed the presence of F1 antigen by fluorescent antibody test (Figure 5).

SPECIAL SECTION: THE PLAGUE EPIDEMIC OF 1994

Table 1. Summary of bacteriological (*Y. pestis*) isolation and identification experiments carried out on samples from Surat city and Beed district

Sample collection			Number processed	Number positive			
Place	Period	Source		Biochemical tests	Phage lysis	F1-FAT	F1-CIE
Surat city	Sept.-Oct. 1994	Human	82	14	11	4*	11
Surat city	Oct.-Nov. 1994	<i>Rattus rattus</i>	4	nil	nil	NT	nil
Surat city	Jul.-Aug. 1995	<i>Rattus rattus</i>	17	1	1	NT	1
Beed dist.	Aug.-Nov. 1994	<i>Rattus rattus</i>	30	4	3	NT	2
Beed dist.	Apr.-May 1995	<i>Rattus rattus</i>	11	1	1	NT	1
Beed dist.	Jul.-Aug. 1995	<i>Rattus rattus</i>	53	1	1	NT	1
		<i>Tatera indica</i>	17	1	1	NT	1

*Only four samples were tested.
NT = Not tested.

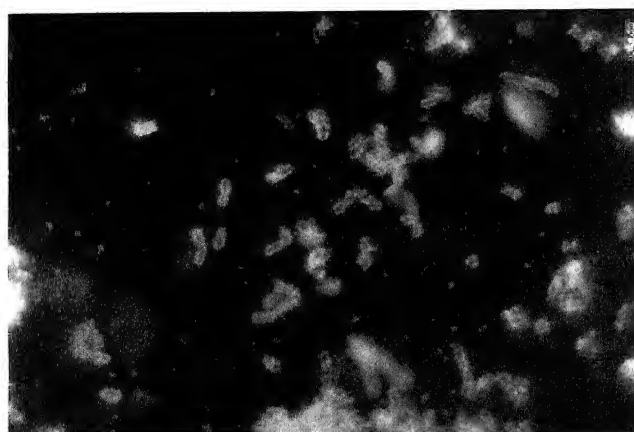


Figure 5. Presence of F1 antigen in a clinical isolate of *Y. pestis* tested by FAT.

Table 2. Age and sex of pneumonic patients yielding *Y. pestis* isolates

S. no.	Date of admission	Age (years)	Sex
1	01.10.94	2	F
2	22.09.94	25	M
3	29.09.94	9	M
4	22.09.94	11	M
5	22.09.94	19	M
6	22.09.94	24	M
7	22.09.94	34	M
8	22.09.94	18	M
9	22.09.94	60	F
10	22.09.94	33	M
11	22.09.94	18	M

17 of the 18 isolates exhibited clear precipitin bands against rabbit anti F1-antibodies. Isolate no. 12 did not show the presence of F1 antigen by this test. Similarly other non *Y. pestis* organisms including the *Y. pseudotuberculosis* and *Y. enterocolitica* were negative.

Discussion

Confirmation of a plague outbreak is achieved chiefly by isolation of *Y. pestis* or by demonstrating the serological responses compatible with the infection. In the present suspected outbreaks in Maharashtra and Gujarat states, the isolation work described here was undertaken after the Technical Advisory Committee was established and after the outbreaks had abated. The samples were collected by our collaborating institutions from patients in Surat at the time of the outbreak. There was a gap of over 45 days from collection to processing of samples from Surat patients. This could have been the main reason for finding mixed growth of organisms in all the 82 samples that were processed and for colonies resembling *Y. pestis* that could be purified only from 11 of these samples. Tissue specimens from 7 out of 132 rodents collected during and after the outbreaks and tested 10 to 30 days after field collection yielded *Y. pestis* organisms following purification from the mixed growth. Although *Y. pestis* grows easily on most of the bacteriological media, the growth is relatively slow. Problems are encountered in differentiating *Y. pestis* when other organisms are also present in the samples. Contaminating organisms are nearly always present in sputum samples and in most of the animal and flea specimens submitted from the field. Cultures showing mixed flora (including *Y. pestis*) on solid or broth media require the microbiologist to proceed promptly within 2-4 days to separate and identify *Y. pestis*. Otherwise, most contaminating organisms by virtue of more rapid growth become so predominant that it becomes difficult to obtain separate colonies of *Y. pestis*. Eventually the contaminants outgrow *Y. pestis* in the culture, making it impossible to confirm the latter's presence⁶. The long gap in processing of specimens from the time of collection is probably responsible for the smaller number of isolates from the Surat patients.

A striking observation was that 9 of the 11 patients from whom the *Y. pestis* was isolated were admitted to

the hospital on 22 September 1994. In accordance with the epidemiological information available, the outbreak in Surat affected mainly young adults and started on 19 September 1994. The clinical diagnosis of suspected pneumonic plague was made on 21 September 1994. Since at that stage therapy with antibiotics appropriate for plague had just been initiated, this could have contributed to a certain extent in the recovery of more isolates from these early cases. The maximum number of isolations (9) were from male patients in the young age group of 9 to 34 years.

It has been stated that positive results obtained by Gram, Giemsa or Wayson staining of smears to identify safety-pin appearance of *Y. pestis* and the F1-FAT test are not confirmatory for plague but are vital preliminary steps in making the diagnosis^{7,8}. In our study, Gram and Wayson staining for safety-pin appearance was quite confusing even to arrive at a presumptive diagnosis. The F1-FAT was not done directly on clinical samples from the present outbreaks because of a scarcity of the necessary reagents.

Biochemical characterization using the above-mentioned biochemical tests was not conclusive to establish a firm identity. Four of 22 isolates which biochemically resembled *Y. pestis* turned out to be different organisms when SDS-PAGE technique for protein profiles was undertaken. These were also not lysed by specific bacteriophage.

Among the rodent isolates, 3 were from affected areas in district Beed from specimens collected within a period of 3 months after the outbreak. The remaining 3 from the same areas were recovered within a period of 9 months to one year after the outbreak. One among these was from *Tatera indica*, the pericommensal rodent, trapped within one kilometer radius of Mamla village with a flea index of 5.5. The lone Surat rodent isolate was from Ved Road locality, the most affected area during the outbreak in 1994. These rodent isolations are a pointer to the fact that the plague foci are still active in the two affected areas.

Earlier studies⁹ have shown that *Rattus rattus* and *Rattus exulans* are susceptible commensal rodent species. In the present instance, 6 of the 7 rodent isolates were from trapped *Rattus rattus* specimens. Probably the *Y. pestis* infection in these animals did not reach the stage of being fatal or the infecting organisms are less virulent or may be the *Rattus rattus* in these areas are becoming relatively resistant. The presence of F1 capsular antigen, a virulent marker, was, however, demonstrated in 6 of the 7 isolates by CIE and the existence of 70 kb plasmid was confirmed in all the isolates.

It is well known that once plague becomes endemic in foci in a country, it is very difficult to eliminate it because the rodent reservoirs of these organisms cannot be eliminated. Regular monitoring of rodent populations and routine testing of suspected patients should form important components in plague surveillance systems. Awareness of the fact that apart from bubonic or pneumonic appearances, gastrointestinal symptoms have been frequently recorded as primary clinical manifestations of confirmed *Y. pestis* infection¹⁰ requires the clinicians to be extra cautious. Abdominal pain may be attributed to intra-abdominal buboes, which can occur without inguinal lymph node involvement⁷. In a recent case study report, confirmed plague presented itself as upper respiratory tract infection, nonspecific febrile syndrome, gastrointestinal or urinary tract infections and meningitis¹¹. Therefore, for the differential diagnosis of such syndromes, a reliable diagnosis of plague is essential.

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Passive haemagglutination tests for *Y. pestis* infection in Surat pneumonic patients

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Passive haemagglutination tests, utilizing a highly purified, specific Fraction 1 antigen of *Yersinia pestis* have been employed to detect antibodies in the sera of pneumonic patients of Surat city. The high antibody titres observed in a majority of sera samples (85.2%) from convalescent patients 2 to 3 months following the outbreak are indicative of *Y. pestis* infection.

SEROLOGICAL responses against the *Y. pestis*-specific Fraction 1 (F1) antigen have been successfully employed for detection of plague in serosurveillance studies. Even at the time of outbreaks, serological tests have their merits in providing confirmatory evidences of plague infection particularly so when the bacterial isolations have been neglected. Passive haemagglutination/passive haemagglutination inhibition tests (PHA/PHAI) and complement fixation test employing the highly purified, specific Fraction-1 antigen of *Y. pestis* are commonly used to detect antibodies in plague¹⁻³. Plate- and dot-enzyme linked immunosorbent assays (ELISAs) for detection of antibodies to F1 and a monoclonal antibody based ELISA for F1 antigen detection have been described⁴⁻⁶. The WHO recommended tests are PHA and PHAI and these are reported to have a high degree of specificity and sensitivity for detection of anti F1 antibodies⁷.

The serum samples from human patients, rodents and other animals following suspected bubonic plague in district Beed and suspected pneumonic plague outbreaks in Surat city were tested at various intervals by several institutions namely, the National Institute of Communicable Diseases (NICD), The National Institute of Virology (NIV) and WHO. The present work reports the antibody test results on serum samples collected from convalescent patients from the outbreak-affected areas.

Materials and methods

Serum samples

A batch of 27 convalescent serum samples from patients with suspected pneumonic plague from Surat were collected in the last week of December, 1994, two to three months after the outbreak. Another batch of 102 serum

samples were collected from Surat city during the month of March, 1995.

Eighteen serum samples collected from Beed district of Maharashtra in the month of April, 1995 and 13 serum samples during July, 1995 were also tested. These human serum samples were from the general population of that area.

A group of 55 human serum samples of pyrexia cases (pyrexia of unknown origin, PUO) negative for typhoid and malaria infections collected prior to the suspected plague outbreaks from a non-affected area were also tested simultaneously.

PHA and PHAI tests

The sera were examined for antibody to the F1 antigen using the PHA and PHAI microtitration procedures recommended by WHO for the serodiagnosis of plague^{8,9}. Briefly, serum samples were heat inactivated at 56°C for 30 minutes and then 50 µl of packed, washed sheep red blood cells (SRBC) added to every 0.5 ml of test serum. They were adsorbed for 60 minutes at room temperature and then centrifuged at 1500 rpm for 10 minutes. For PHA test, 25 µl of PHA diluent (1% normal rabbit serum in normal saline) was added to all wells of 'U'-shaped microtitration plates. For PHAI test, the diluent used had 0.2 mg/ml F1 antigen in PHA diluent. In the next step, 25 µl of test serum was added to the first well of microtitration plate and serially diluted. A volume of 25 µl of appropriately diluted F1 sensitized SRBC was added to all the wells. Plates were covered and incubated at room temperature for 6 h and then overnight at 4°C.

Results

The wells were examined for flocculent, lattice agglutination. Negative wells showed a sharp-edged button or a defined circle. End-point titres were determined by taking the last well showing complete agglutination. Final titres were determined by subtracting PHAI titres from PHA titres of individual sera samples. Among the first batch of 27 samples from suspected convalescent plague patients from Surat, 23 showed titres of 1:16 or more

Table 1. PHA and PHAI titres of tested human sera samples

Place	Period of collection	Total tested	Number with titres								Total positive (%)
			Nil	1:4	1:8	1:16	1:32	1:64	1:128	1:256	
Surat	Dec. '94	27	—	—	4	2	12	7	2	—	23 (85.2)
Surat	March '95	102	77	6	10	2	3	2	1	1	9 (08.8)
Beed	April '95	18	12	3	3	—	—	—	—	—	—
Beed	July '95	13	11	1	1	—	—	—	—	—	—
Gwalior	July '94	55	52	—	1	1	1	—	—	—	2 (03.6)

(Table 1). Of the 102 sera samples collected from Surat in the second batch, only 9 showed positive titres. Human serum samples collected 8 to 11 months after the outbreak in district Beed were found to be negative.

Two of the 55 sera samples of PUO cases from non-affected area were also positive with titres of 1:16 and 1:32, respectively.

Discussion

Confirmation of a clinical diagnosis of plague is based chiefly on isolation of *Y. pestis* and/or demonstration of four-fold rise of antibody titres at paired sera testing. It is well established that *Y. pestis* antibody is more readily detected than isolation and identification of *Y. pestis*.

Confirmation of plague by serology, particularly in pneumonic cases, is retrospective to the outcome for the patient as most fatalities occur within a week of infection, whereas, antibodies generally appear in significant titres about one to two weeks following the infection. Therefore, antibody detection from patients during the period of acute illness is of limited value. Serology on convalescent patients, two weeks to few months after infection is more suggestive of plague occurrence. A serologic titre of 1:10 or more in the absence of known recent plague vaccine history is considered positive for plague antibodies. A four-fold rise in titre between two time distanced samples (greater than two weeks apart) is considered confirmatory⁸. Since acute phase serum samples collected during the outbreak were not available for testing, it was not possible to analyse results of paired sera testing. Serological results on blood samples collected from convalescent patients 2–3 months following the pneumonic outbreak were, therefore, analysed independently. A majority of cases (23 out of 27) showed high levels of positive titres (>1:16 to >1:256). Such a large proportion of convalescent cases showing positive PHA and PHAI titres against specific F1 antigen of *Y. pestis* is highly suggestive of plague in pneumonic patients of Surat city. Only two of the 55 sera samples from the non-affected area had positive titres. Detection of these two positive cases from among the PUO cases from the non-affected area and that too, prior

to the appearance of the outbreak is enigmatic. Could it be due to the presence of *Y. pestis* infection in an inapparent form in that region or a cross-reactivity with other disease-causing agents? This question needs further study.

Serum samples collected 5–6 months after the outbreak showed a low percentage of positive titres. A progressively decreasing number of positive serological reactions within a period of several months is indicative of effective control of the outbreak⁷.

Human serum samples from district Beed were all found negative for antibodies to F1 antigen. Since the sample collection from the individuals was after 8–11 months of suspected plague outbreak in that area, probably the antibody titres by this time had disappeared. Moreover, these samples were from general population and more likely to be the contact individuals rather than the patients who suffered with suspected bubonic plague illness.

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The 1994 plague epidemic of India: Molecular diagnosis and characterization of *Yersinia pestis* isolates from Surat and Beed

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PCR analysis of formalin-fixed human autopsy tissues, rodent tissues, fleas and bacterial isolates from pneumonic patients from the 1994 plague epidemic confirmed the presence of the *f1* and *pla* genes of *Yersinia pestis* in these samples. Several *Y. pestis* isolates from the epidemic areas were studied in respect of their plasmid profile, expression of F1 antigen and ribotype pattern. All the three plasmids known to be associated with virulence were present in the Surat isolates of *Y. pestis*. Presence of the F1 antigen, classically used for diagnosis of *Y. pestis* infection, was demonstrated by immunoblotting. All the Indian isolates from the 1994 epidemic showed an identical ribotype profile. This profile, however, was different from those of *Y. pestis* isolates tested from other regions of the world.

Upon digestion with *EcoRI* and *EcoRV*, and probing with *E. coli* 16S and 23S rRNA genes, DNA from these *Y. pestis* isolates gave two distinct profiles which, taken together, suggest that the present Indian isolates represent a new ribotype. The presence of *Y. pestis* signature genes in 5 out of 7 fleas collected from rodents in the affected areas, and the occurrence of the same ribotype in the *Y. pestis* isolates from domestic rodents, sylvatic rodents and the patients are strongly indicative of a clonal origin of this Indian strain and an epidemiological linkage among wild rodents, domestic rats and humans in the epidemic area.

IN August 1994 the village Mamla in Beed district of Maharashtra state experienced an unusually heavy flea nuisance and rat falls. Subsequently, a number of cases clinically resembling bubonic plague were reported from Mamla and nearby villages of Beed district. Based on the totality of ecological, clinical and serological

evidence, a presumptive diagnosis of bubonic plague was made and the health machinery of the State Government, with the active support of the Central Government, took prompt measures and the epidemic was successfully contained.

In September 1994, Government hospitals and private clinics in the city of Surat in the neighbouring state of Gujarat reported an increasing number of patients with an illness resembling acute pneumonia. Based on clinical, laboratory and radiological findings, a presumptive diagnosis of an outbreak of pneumonic plague was made.

Plague is an acute zoonotic bacterial disease caused by infection with *Yersinia pestis*. *Yersinia* belongs to a group of bacterial pathogens of the family Enterobacteriaceae. Three members of this group, *Yersinia pseudotuberculosis*, *Yersinia enterocolitica* and *Yersinia pestis*, are human pathogens. These species carry one or more plasmids of molecular weights 110 kb, 70 kb and 9.5 kb which have proved to be of diagnostic value. Only *Yersinia pestis* carries all the three plasmids^{1,2}.

The pathogenicity in *Y. pestis* is multifactorial, with the genes responsible being present on the chromosome as well as on all the three plasmids. The 9.5 kb plasmid is unique to *Y. pestis* and is not present in the other two *Yersinia* species. This plasmid carries the *pla* gene which codes for a virulence factor called plasminogen activator. The complete sequence of this 1041 nucleotide-long gene is known³. Although *pla* has partial sequence homology to the '*omp*'-T gene of *E. coli* and gene '*e*' of *Salmonella typhimurium*, there are unique regions which can be used for amplification of the *pla* gene by PCR for identification of *Y. pestis* isolates⁴ and plague surveillance⁵. The two other plasmids, 110 kb and 70 kb, produce specific products like Fra I and

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Table 1. Source and place of origin of the *Y. pestis* isolates used in this study

Isolate no.	Source	Place	Date of collection	Nature of specimen
4	Human (pneumonic plague)	Surat	Sept./Oct. 1994	Sputum
8	Human (pneumonic plague)	Surat	Sept./Oct. 1994	Sputum
9	Human (pneumonic plague)	Surat	Sept./Oct. 1994	Sputum
101	Human (pneumonic plague)	Surat	Sept./Oct. 1994	Sputum
102	Human (pneumonic plague)	Surat	Sept./Oct. 1994	Sputum
103	Human (pneumonic plague)	Surat	Sept./Oct. 1994	Sputum
104	Human (pneumonic plague)	Surat	Sept./Oct. 1994	Sputum
105	Human (pneumonic plague)	Surat	Sept./Oct. 1994	Sputum
106	Human (pneumonic plague)	Surat	Sept./Oct. 1994	Sputum
107	Human (pneumonic plague)	Surat	Sept./Oct. 1994	Sputum
108	Human (pneumonic plague)	Surat	Sept./Oct. 1994	Sputum
111	<i>Rattus rattus</i>	Village Mamla	May 1995	Liver and Spleen
112	<i>Tatera indica</i>	Village Mamla	Aug 1995	Liver and Spleen
113	<i>Rattus rattus</i>	Surat	Aug 1995	Liver and Spleen
114	<i>Rattus rattus</i>	Village Mamla	May 1995	Liver and Spleen
115	Rodent (species ?)	Village Mamla	Nov 1994	Liver and Spleen
116	<i>Rattus rattus</i>	Village Mamla	Nov 1994	Liver and Spleen
117	<i>Rattus rattus</i>	Village Mamla	Aug 1995	Liver and Spleen

Yersinia Yops protein. The *Fra1* protein, encoded by *fl* on the 110 kb plasmid, is unique to *Y. pestis* and has been classically utilized for diagnosis of *Y. pestis* infection. This gene is highly conserved amongst various isolates of *Y. pestis* and thus provides the characteristic 'signature' for *Y. pestis*.

The present report describes results which demonstrate the presence of *Y. pestis* genes in human tissue samples collected at autopsies in Surat. The same signature sequences were demonstrated in fleas collected from rodents trapped during the epidemic in Beed. Molecular characterization of *Y. pestis* isolates from Beed and Surat is also described.

Materials and methods

Bacterial cultures. The bacterial isolates (Table 1) were grown from single colonies in nutrient broth for 24 hours. The cells were pelleted, resuspended in 70% ethanol and stored at -20°C until further use.

Formalin-fixed human tissues. Tissue samples from lungs and spleen were collected at autopsy from deceased persons and stored in 10% buffered formalin. All patients were from Surat with pre-mortem diagnosis of pneumonic plague.

Fleas. Fleas were collected from the epidemic areas in Beed district and stored in 70% ethanol.

Polymerase chain reaction

Primers. The primers used for PCR are listed in Table 2. These were synthesized on an automated oligonucleotide

Table 2. Sequences of oligonucleotide primers used in this study

For the plasminogen activator gene:

YP1-5' ATCTTACTTTCCGTGAGAAG 3'
 YP2-5' CTTGGATGTTGAGCTTCCTA 3'
 YP3-5' ATACTGTGACGGCGGGTCTG 3'

For the *fl* gene:

FI-1-5' ATACTGCAGATGAAAAAATCAGTTCC 3'
 FI-2-5' ATAAAGCTTTTATTGGTTAGATACGGT 3'
 FI-1405' GCCCGCATCACTCTTACATA 3'
 FI-3845' CATCCCCACAAGGTTCTCA 3'

synthesizer (Applied Biosystems, USA; Model 392) using phosphoramidite chemistry.

DNA preparation. The bacterial cells stored in 70% ethanol were centrifuged at 10,000 rpm for 5 minutes. The supernatant was discarded, the pellet was air dried and resuspended in 100 µl of sterile distilled water. The samples were boiled for 10 minutes and rapidly chilled on ice. The boiled material was centrifuged at 10,000 rpm for 5 minutes and the supernatant was used as template for PCR.

Approximately 10 mg of tissue was sliced from each of the formalin-stored samples and washed thrice with 0.05 M tris-HCl (pH 7.5). The material was ground in 50 mM tris-HCl (pH 8.0), 50 mM KCl, 2.5 mM MgCl₂, 0.45% Tween-20, 0.45% NP-40, using a glass rod. Proteinase K digestion was carried out overnight at 55°C in 500 µl of buffer (10 mM tris-HCl, 5 mM EDTA and 0.5% SDS) containing proteinase K (100 µg/ml). The samples were then extracted with hot phenol for 2 hours at 65°C. After chloroform extraction, the supernatant was precipitated at -70°C with 0.3 M sodium acetate and two volumes of ethanol. The extracted DNA was

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pelleted and washed twice with 70% ethanol. The pellet was air-dried and reconstituted in 25 µl sterile distilled water. The samples were denatured for 5 min in boiling water and then chilled in ice.

Ethanol was drained from the flea samples which were then air dried. DNA was extracted by the hot phenol method as described.

Both positive and negative controls were included and all possible precautions were taken to avoid contamination.

Amplification of DNA by PCR. For amplification of the *pla* gene, PCR was carried out in 100 µl reaction volumes with 50 pmol each of external primers (Table 2), 1X Taq buffer, 3 mM MgCl₂, 400 µM dNTPs, 2.5 units of Taq DNA polymerase (Stratagene) and 1/10th volume of template DNA. The reactions were carried out by programming the DNA thermocycler (Technique, UK) at 95°C for 5 min, 52°C for 1 min and 73°C for 1 min for 1 cycle followed by 2 cycles at 95°C for 2 min, 52°C for 1.3 min and 73°C for 1.3 min; 35 cycles at 95°C for 1 min, 51°C for 1 min and 73°C for 1.3 min. One tenth volume of the first PCR product was reamplified with internal primers using the same conditions. For amplification of *f1*, the reactions were set up as above except that 1.5 mM MgCl₂ was used. The reactions were carried out at 95°C for 5 min followed by 35 cycles at 95°C for 1 min, 51°C for 1 min and 72°C for 2 min.

Ribotyping

Bacterial cells were grown in nutrient peptone broth from single colonies for 48 h. The cells were pelleted and chromosomal DNA was extracted using SDS-Proteinase K digestion followed by phenol-chloroform extraction. DNA samples (2.5 µg each) were digested with *EcoRI* or *EcoRV* restriction enzymes (Promega, USA) for 4 h at 37°C according to manufacturer's instructions. Electrophoresis was carried out on 0.8% agarose gel in 1X TBE buffer (90 mM Tris, 9 mM boric acid, 2 mM EDTA, pH 8.0) overnight. Southern hybridization was performed using Hybond membranes (Amersham, England) essentially according to the manufacturer's instructions. Fragments carrying the 16S, 23S and 5S *E. coli* rRNA genes⁶, from plasmids pKK 3535/pL215 were used as probes. In addition, 16S and 23S ribosomal RNAs were purified from *E. coli* and used as probes. The probes were ³²P-labelled by nick translation or by 5' end labelling. PCR ribotyping was carried out using primers from the conserved regions of the 16S rRNA gene.

Plasmid analysis by pulsed field gel electrophoresis (PFGE)

The bacterial cultures were grown overnight in BHI broth from single colonies. Cells from 1.5 ml of culture

were pelleted and the plasmids isolated using commercially available kits (Wizard, Promega, USA). Purified plasmids were suspended in 100 µl of Tris-EDTA buffer (pH 8.0) and mixed with an equal volume of 2% low-melting agarose. The plugs were prepared in Biorad moulds and subjected to PFGE in 1% agarose in 0.5X TBE buffer for 21 hours at 18°C. Lambda oligomers were used as size markers. The plasmids were visualized after staining with ethidium bromide.

Protein profile studies using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Bacterial cells were grown overnight on BHI agar from single colonies. A loopful of cells was suspended in PBS, washed thrice in the same buffer and pelleted. The pellets were resuspended in 50 µl distilled water and 50 µl SDS-PAGE loading dye. The samples were boiled for 4 minutes at 100°C and run in a 10% polyacrylamide gel at 40 mA until the dye front was close to the bottom of the gel. The protein bands were visualized by staining with Coomassie blue using standard protocols.

Immunoblot analysis for F1 antigen expression

The bacterial cell lysates were subjected to electrophoresis in 10% SDS-PAGE as above and the proteins were transferred onto nitrocellulose membranes (S&S, Germany), at 100 mA for 1 hour. The membranes were blocked with 3% BSA overnight and incubated with rabbit anti-F1 antibodies at a dilution of 1:20,000 for 1 hour. The F1 antigen was detected with alkaline phosphatase-conjugated anti-rabbit IgG (1:6,000 dilution) for 1 hour. It was then developed with phosphatase substrate for 4–5 minutes.

All experiments reported here were performed independently in more than one laboratory.



Figure 1. PCR amplification of *pla* gene fragments (478 bp) from bacterial isolates. Lane 1 – positive control; 2 – negative control; 3 – *Y. pestis* isolate from human pneumonic patient, Surat; 4 – *Salmonella typhi*; 5 – *E. coli*; 6&7 – *Y. pestis* isolates from two different pneumonic plague patients, Surat; 8 – *Klebsiella pneumoniae*, 9&10 – *Y. pestis* isolates from two pneumonic plague patients, Surat; 11 – *Proteus* sp., 12 – *Y. pestis* isolate from plague patient, Surat; 13 – *Streptococcus pneumoniae*; 14 – *Y. pestis* isolate from plague patient, Surat; 15 – *H. influenzae*.

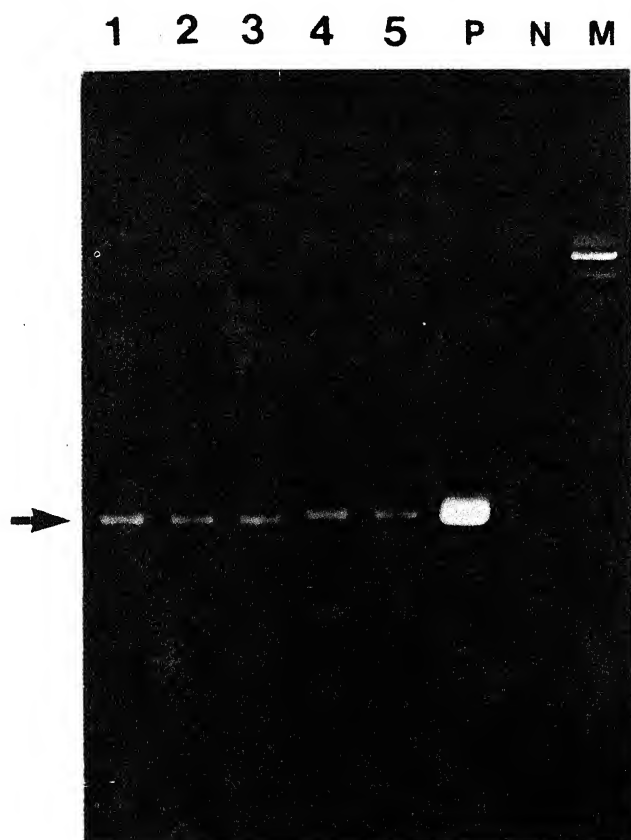


Figure 2. PCR amplification of *f1* gene fragments (274 bp) from autopsy samples. Lane 1 – A1/94, 2 – A6/94, 3 – A4/94, 4 – A10/94, 5 – A13/94. P, positive control A1122 *Y. pestis*; N, negative control; M, molecular weight marker.

Table 3. Detection of *pla* and *f1* genes in autopsy tissues using the polymerase chain reaction

Source	Autopsy ID no.	<i>pla</i> gene	<i>f1</i> gene
Bhula Bhai	A21/94	–ve	NT
Fulmani Loknath	A20/94	–ve	NT
Chandu Bhai	A1/94	+ve	+ve
Guruji Bhai	A6/94	+ve	+ve
Dinesh Bhai	A4/94	–ve	+ve
Chirag	A10/94	+ve	+ve
Vedi Ben	A13/94	–ve	+ve

NT – not tested.

Results

Conditions for PCR amplification of the *pla* and *f1* genes were standardized. DNA samples isolated from a number of bacteria pathogenic to humans, but known to lack the *pla* and *f1* genes, were used as controls in the PCR experiments. DNA samples from the 18 *Y. pestis* isolates from the epidemic areas (Table 1) showed the presence of both the *pla* and *f1* genes. Of the 7 autopsy tissue samples, 3 were positive for the *pla* gene (Table 3) and 5 were positive for the *f1* gene (Figure 2 and Table 3). Five of the 7 flea samples (C1, C2, C3, ANJ4

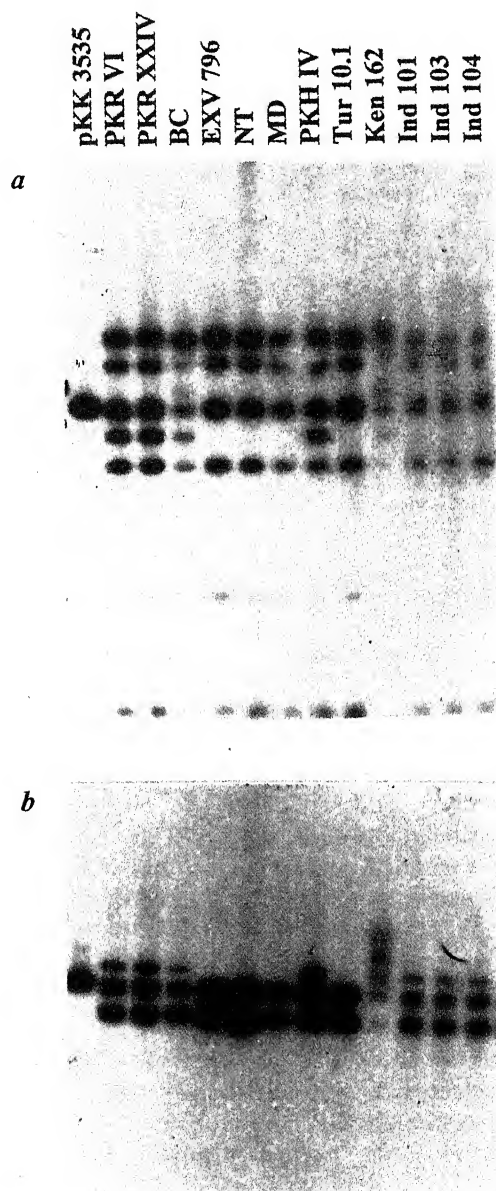


Figure 3. Southern hybridization of *EcoRI*- and *EcoRV*-digested *Y. pestis* chromosomal DNA. ³²P-labelled *E. coli* 16 S and 23 S rDNAs derived from pKK3535 were used as probes. Panel A indicates *EcoRI* digests and Panel B, *EcoRV* digests. Names of *Y. pestis* strains tested are indicated at the top. Patterns for only a few Indian isolates are shown. None of the Indian isolates (Ind 101, 103 and 104) has a profile similar to any other *Y. pestis* strains tested.

and ANJ6) collected from Beed during the epidemic period were positive for the *f1* gene (data not shown). The amplified products were cloned and sequenced and the identity of the genes confirmed. The *pla* and *f1* genes did not amplify in any of the control samples.

For ribotyping of the *Y. pestis* isolates from Surat and Beed, restriction endonucleases *EcoRI* and *EcoRV*, which are known to be the most discriminatory⁷, were used. A few strains isolated from different parts of the world and already ribotyped at the WHO *Yersinia*

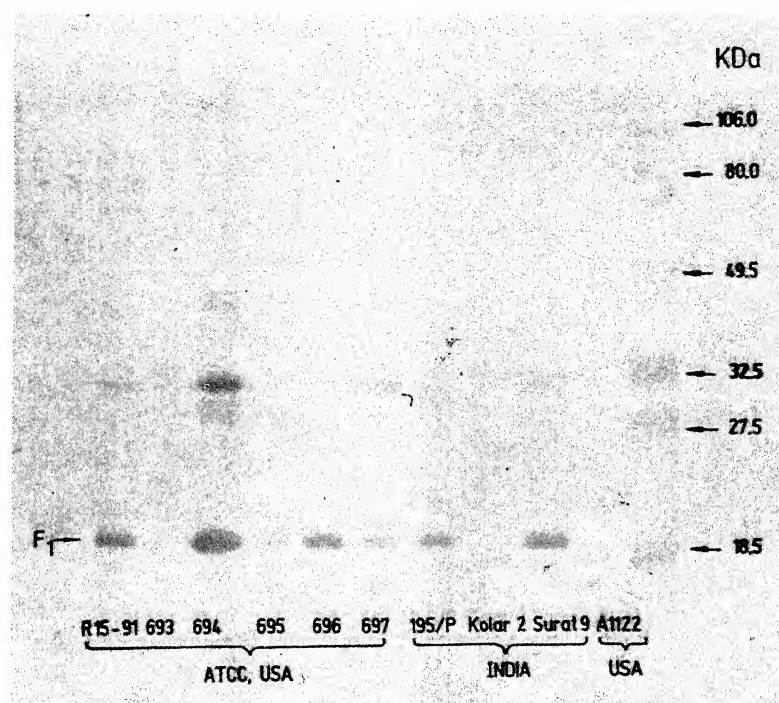


Figure 4. Western blot analysis of *Y. pestis* isolate no. India-9 (from Surat) in comparison with the other older Indian isolates and controls showing expression of F1 antigen of ~19 kDa.

Reference Centre, Pasteur Institute, Paris, were included in this study.

EcoRI ribotype profiles displayed by the Indian isolates were identical to one another and were similar to those displayed by strains EXV 796, Nhatrang, Madagascar and Turkey 10.1 but differed from those displayed by strains PKR VI, PKR XXIV, Belgian Congo, PKH IV and Kenya 162 (Figure 3). When an identical set of experiments was performed with the restriction endonuclease *EcoRV*, it was observed that, again, all Indian isolates had the same ribotype profile (Figure 3). Unlike the *EcoRI* case, however, this profile differed markedly from the profiles displayed by EXV 796, Nhatrang, Madagascar and Turkey 10.1 but were similar to those displayed by PKR VI, PKR XXIV, Belgian Congo and PKH IV (Figure 3). Of particular interest is the fact that when both *EcoRI* and *EcoRV* ribotype profiles are considered together, the Indian isolates had profiles distinct from those of the foreign isolates, (Figure 3). This combination of *EcoRI* and *EcoRV* patterns seen in the present Indian isolates has not been seen in any of the *Y. pestis* strains examined earlier (Guiyoule *et al.*⁷ and personal communication⁸). Thus, the ribotype observed in this study constitutes a new ribotype, named 'S' for Surat by the WHO *Yersinia* Reference Centre, Pasteur Institute, Paris. All the present Indian isolates were found to belong to this ribotype. Similar ribotype patterns were obtained irrespective of the type of probe used. Upon PCR amplification of the 16S rRNA gene, products of identical size were obtained from all isolates originating from Beed and Surat.

Pulsed field gel electrophoresis carried out on three isolates (4, 8, 9) demonstrated the presence of all the three plasmids.

In SDS-PAGE, the overall migration pattern of the proteins was similar in all the Indian isolates. A protein band of approximately 25 kDa was observed in the Surat isolates 4, 8 and 9 at the WHO Reference Centre, CDC, Fort Collins, USA. However, our own attempts to reproduce this result were unsuccessful (see Discussion below).

The F1 antigen was detected in all the isolates by immunoblot assay, indicating that the isolates were pathogenic (Figure 4 shows results for isolate-9).

Discussion

After the outbreaks in Beed and Surat, it was obviously necessary to identify and characterize the causative organism. This was achieved by PCR analysis of autopsy tissues from 7 individuals using primers for the *Y. pestis*-specific *pla* and *f1* genes. Five out of the 7 individuals whose autopsy tissues were analysed were positive for the *f1* gene and three were also positive for *pla*, providing unequivocal evidence of the involvement of *Y. pestis* in these deaths. It is worth mentioning that this is perhaps the first time that such *in situ* demonstration of *Y. pestis* in historical material had been attempted. By PCR analysis, eighteen bacteriologically characterized *Y. pestis* isolates were shown to be positive for the *Y. pestis* *pla* and *f1* genes (Table 1). Eleven of these isolates were from pneumonic plague patients from Surat city and seven were from rodents collected from the

epidemic areas during the period August 1994 to August 1995.

Numerous reports have shown that ribotyping is an extremely useful tool for the molecular characterization of bacterial species. To differentiate strains belonging to the same species by ribotyping, it is essential to choose restriction endonucleases which are most discriminatory. Guiyoule *et al.*⁷ have demonstrated that for the analysis of *Y. pestis* strains, restriction endonucleases *EcoRI* and *EcoRV* are particularly useful. *Y. pestis* strains isolated from Surat and Beed (including those isolated from trapped rodents) showed the same ribotype profile. Further, this profile (i.e. *EcoRI* and *EcoRV* patterns considered together) differed from those obtained with all other isolates examined so far (Guiyoule *et al.*⁷ and personal communication⁸), justifying the conclusion that these Indian strains are clonal in origin. Among the rodent isolates studied, one was from a *Tatera indica* specimen, a sylvatic species, and six were from *Rattus rattus*, a domestic species. The ribotype profile observed in the bacterial isolates from these domestic and sylvatic rodents was the same as that observed in the isolates from the patients. This is strongly supportive of an epidemiological linkage among wild rodents, domestic rats and humans in the epidemic area. The observation that five out of the seven flea samples collected from trapped rodents in the affected area also showed the presence of *Y. pestis* signature genes is in support of such an epidemiological connection.

The 85 *Y. pestis* strains examined by Guiyoule *et al.*⁷ have been classified into 16 ribotypes⁷. Even though the ribotype of the Surat strain is distinct from the 16 previously known ribotypes, it does not necessarily mean that it defines a strain of recent origin. This is because, as mentioned above, ribotyping has been done on only 85 out of the approximately 6000 strains known to exist in *Y. pestis* collections worldwide. Therefore, it is entirely possible that among the remaining 5900 or so strains, the Surat ribotype could be present in one or more strains. This can be only ascertained by further investigation.

Even though one of the worst pandemics in history occurred in India, and about 12 million people died between 1896 and 1930, only a few strains from these years have survived in various culture collections, mostly outside India. Considering that the only isolate ribotyped from India was collected in 1908 and that very few other Indian isolates are available over the last 100 years, it is not possible to say whether the Surat strain is of recent origin. This is because of our ignorance of the ribotype characteristics of the large number of strains that must have existed in the country during the past 90 or so years.

Studies carried out by the Pasteur Institute, Paris on *Y. pestis* isolates from Madagascar indicate the presence of more than one ribotype in this small island country (E. Carniel, personal communication). Moreover, there is a specific geographical distribution of these variants on

the island. Drug-resistant strains of *Y. pestis* have reportedly arisen recently in this endemic area.

The presence of all the three plasmids characteristic of *Y. pestis* in the Surat isolates was confirmed by pulsed field gel electrophoresis. This observation suggests that the Surat strain is different from the Russian vaccine strain EV because the latter lacks one of the three plasmids.

Upon single dimension SDS-PAGE, the protein profiles of the different *Y. pestis* strains studied by us showed an overall similarity. However, in Surat strains 4, 8 and 9, an additional 25 kd protein band was observed in experiments done at the WHO Reference Center, CDC, Fort Collins, USA. This band was not detected in subsequent studies done by us on the same isolates. This is perhaps not surprising in view of the fact that protein profiles obtained by single dimension SDS-PAGE can produce non-identical patterns depending upon the culture conditions, degradation of high molecular weight proteins and other variables. Even in case of *Y. pestis* it has been observed that though there is an overall similarity in the protein profiles produced by different strains, several display one or more unique bands.

The presence of the F1 antigen, which is routinely used for serodiagnosis of *Y. pestis*, was demonstrated in Surat isolates by immunoblotting, confirming that these isolates were indeed *Y. pestis*.

In conclusion, these results (i) confirm the association of *Y. pestis* with the epidemic at Surat and Beed; (ii) demonstrate that the *Y. pestis* isolates obtained from these regions were identical, indicating that the pathogen was most likely clonal in origin; and (iii) suggest that the pathogen has had an enzootic existence in the region. Further epidemiological studies, as well as collection and characterization of *Y. pestis* isolates from different parts of the Indian subcontinent, are necessary for a better understanding of the dynamics of this important pathogen.

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Ecology of flea-transmitted zoonotic infection in village Mamla, District Beed

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Ecological observations were made in the village Mamla and the area around it in Beed district to (i) gather evidence on ecological support system for natural harbourage of rodent species in domestic, peridomestic and feral situations, and (ii) enumerate ecological factors and situations which may have triggered the 1994 outbreak.

Prior to the Maharashtra earthquake of 1993, the ecosystem of Mamla maintained equilibrium densities of domestic rodent (*Rattus rattus*) and their fleas (*Xenopsylla cheopis*). This prevented fulminating epizootics among domestic rats. However, the impact of the September 1993 earthquake in Maharashtra introduced major changes in human ecology at Mamla village which generated unlimited energy inputs (such as foodgrains) for domestic rodents. As a result, there was a gradual, but persistent, growth of *R. rattus* population in the subsequent 8–10 months quiescent period. In August 1994, the *R. rattus* population overshot its equilibrium density level, which eventually led to a flea-mediated epizootic in rats and an outbreak of a cryptozootic, possibly plague, infection in humans in Mamla.

FLEAS as ectoparasites of rodents are known to be the vectors of human diseases like plague, murine typhus and tularemia. Among the 76 species of fleas recorded from the Indian subcontinent¹, the roles of only 3 species viz. *Xenopsylla astia*, *X. brasiliensis* and *X. cheopis* are well known in plague transmission. However, evidence also exists on the involvement of these flea species in the transmission of murine typhus (*Rickettsia typhi*), which is widespread even in rural India where climatic conditions favour survival of rats and their ectoparasites; however the disease is grossly under-reported^{2,3}. These rodent–flea borne infections may remain in enzootic form with rare or accidental human transmission depending upon the extent of man–flea contact. Local ecological factors play a key role in the ectoparasite-mediated spill-over of infection from rodent to man.

During August 1994, the inhabitants of village Mamla in Beed district of Maharashtra State, experienced heavy flea nuisance. Subsequently, a number of cases clinically resembling bubonic plague were reported from the village⁴. During May 1995, field investigations were

undertaken in and around village Mamla to (i) examine the ecological support system (ESS) for the natural harbourage of rodents in and around the village and (ii) elucidate ecological conditions which may have triggered flea-borne epizootic infection among rodents and its spill-over to humans.

Extensive geographical reconnaissance was undertaken within 30 km around the village Mamla to obtain information on terrain, soil types, vegetation and cropping pattern in agricultural fields. Meteorological data were obtained from the Meteorology Department at Pune.

Wonder traps were laid in the village in the evening and picked up the next morning. Rats were retrieved from the traps and fleas were combed out of their body. Blood was drawn directly from the heart to collect serum for serology. Later, these animals were dissected to take out their lung, liver and spleen and these organs were transported in Cary Blair medium for isolation of bacilli at the Defence Research Laboratory, Gwalior.

Beed district falls along the dry-deciduous scrub land zone of Deccan Lavas region of north-western peninsular plateau. It has small-sized sparse trees mainly *Acacia* sp., with scattered thorny shrubs. The narrow stretches of grasslands used as pastures generally remain dry and barren from March to June.

The village Mamla is an old, remote village, 32 km away from Beed town and falls under Primary Health Centre at Kuppa. The village has a small population of about 350 people, who are mainly agrarian by occupation. During September 1993 a major earthquake shook the adjoining districts of Osmanabad and Latur, but mild tremors were also experienced in certain parts of Beed district. Consequently the residents of Mamla village abandoned their permanent homes in fear during October 1993 and resettled in temporary tin shelters erected on the southern and western borders of the village (Figure 1). The fear of recurrence of earthquake prevented the villagers from reoccupying their old permanent houses which were being used exclusively for storing food grains (wheat, groundnut, millet, etc.). The villagers occasionally visited these houses, for procuring the stored foodgrains.

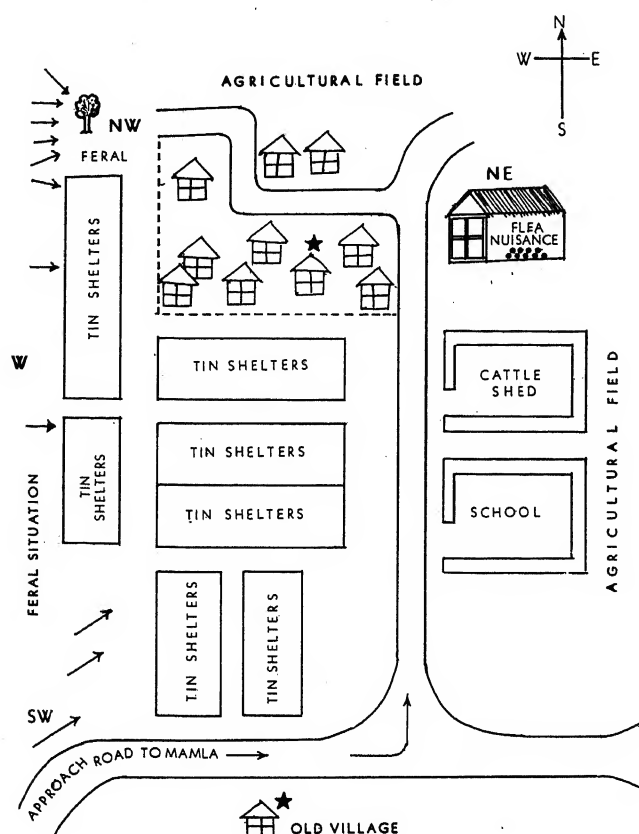


Figure 1. Schematic presentation of village Mamla, Beed.

The village is surrounded on 3 sides, i.e. north, east and south by agricultural fields (agro-climatic zone). However, its western border is contiguous with a small pocket of feral land in the north (Figure 1). The three adjoining borders in the north, east and south of Mamla are cultivated areas, classifiable as a peridomestic agroclimatic zone. The agricultural fields in this zone mainly produce wheat, millet, sugarcane and groundnut. Burrows of the field rodent *Bandicota bengalensis* were found in these agricultural fields mainly in fallow wheat field and a few in the bunds bordering the fields. *B. bengalensis* infestation in the area was relatively poor, though there were some in the fields.

The village has cattle, goat and buffalo populations of about 40, 35 and 18 respectively. Goats are taken every day to open pastures about 1–2 km away for grazing.

The landscape, as observed between Beed city and Mamla village, is a plain and undulating open terrain. The soil is mainly black cotton soil, interspersed with reddish-brown and sandy loose alkaline soil. The black and brown loose soils are mainly used for agriculture but some of the feral areas generally have loose sandy or light red gravelly type of soil. The small discontinuous feral pockets are inhabited by *Tatera indica*.

Dry area crops like millet, cotton, groundnut, maize and wheat are the predominant crops, but in some areas with irrigation facilities sugarcane is also grown.

The maximum temperature during summer touches 44°C in May and the minimum falls to a low of about 5°C in January. However, the monthly mean maximum temperature varies from 29°C to 42°C and the monthly mean minimum temperature varies from 12°C to 25°C. The mean minimum temperature from April to September oscillates between 22°C and 26°C. Beed district is a relatively low rainfall area. An appraisal of monthly rainfall data for the year 1991 indicated that during the monsoon season of 120 days (June to September) there was a total of 20 rainy days, varying from 3 to 9 rainy days per month.

In the domestic biotope of village Mamla, a total of 10 rodents (*Rattus rattus*) was trapped from the peripheral houses of the village and 11 fleas (*Xenopsylla cheopis*) were collected from their bodies. The rodent and parasite indices are as follows:

Trap positivity	16.6%
Flea infestation rate	60.0%
Flea index (<i>X. cheopis</i>)	1.1
Intensity of infestation	1.8

These rats were found to be distributed towards the periphery in the old village and tin shelters in the north, west and southern parts of the village. The central and eastern areas of the village had a sparse rat population.

Organs from 10 *R. rattus* specimens which were processed for *Y. pestis* isolation did not reveal presence of the microorganism and serological tests were also negative for antibodies against *Y. pestis*.

The ecological support system was found to be highly conducive for natural harbourage of *Rattus rattus* in the domestic biotope; *Tatera indica* in the peridomestic feral biotope and *Bandicota bengalensis* in the peridomestic agroclimatic zone.

The biotope association of village Mamla is of a primitive type⁵ wherein the domestic biotope has direct ecological linkages with both the agro-climatic zone and the feral biotope. Such biotope associations are highly favourable for spillover of rodent-borne flea-mediated infection (such as plague) from the wild (feral) directly into the domestic biotope as the (agro-climatic) barrier zone⁵⁻⁷ is absent between the feral and the domestic biotopes on the western border of Mamla. Another known route of spillover of zoonotic infection from wild to domestic situation which was observed in Mamla is by passive transportation of wild fleas (*Xenopsylla astia*) from pasture land (feral) to the domestic through grazing goats. Such passive transportation of fleas has been reported earlier from Karnataka⁶. From the observations in and around village Mamla and the history of earthquake in the area during 1993 and its impact on human

ecology and other associated ecological features of the village, 4 situations were discernible as far as the dynamics of flea-transmitted zoonotic infections was concerned.

Situation I	(Pre-1993 earthquake)
Situation II	(October 1993; impact of earthquake)
Situation III	(September 1993 – July 1994; quiescent period)
Situation IV	(5 August 1995 onwards; outbreak period).

From situation I standpoint, the biotope-associated ecological linkages of the village Mamla in respect of the rodent and flea populations reveal a state of ecological equilibrium that existed in the rodent–flea relationship during the pre-earthquake period. The natural regulation of host and parasite populations was under density-dependent control factors which presumably prevented the flea population from increasing beyond the level necessary to initiate an epizootic or create pestilence among humans. Density-independent factors like temperature and rainfall in Mamla are not generally inimical to the growth of *R. rattus* and its flea population, as is evident from the minimum and maximum temperature range of the area and poor precipitation.

In situation II, the impact of the September 1993 earthquake in Latur and Osmanabad districts was also noticeable in Mamla village where the inhabitants abandoned their homes in October, 1993, leaving behind large quantities of foodgrains in their homes. The residents shifted to temporary tin shelters thereafter.

In situation III (quiescent period), between October 1993 and July 1994 (for about 10 months), the abandoned homes had ample quantities of stored food grains, for consumption by rodents without human disturbance. This period therefore witnessed on exponential growth of rat populations supported by an abundance and easy accessibility of food in the abandoned homes. This silent period, which promoted unrestricted reproduction and growth of *R. rattus* and *X. cheopis* populations between October 1993 and July 1994 is thus a result of imbalance in ecological equilibria.

In situation IV (August 1994), due to unrestricted growth of *R. rattus* and its flea *X. cheopis* for the previous 10 months, an unusual flea nuisance was seen, having been first detected in one of the peripheral houses in

north-eastern corner of the village (Figure 1). It seems probable that the rat population growth which overshot its upper asymptote level sometimes in July–August, 1994 resulted in shortage of space and at this juncture density-dependent population controls, of which disease is a component, operated on the rat population, leading to a rodent epizootic, mediated by the flea *X. cheopis*. A large number of susceptible individuals in the rat population may have suffered mortality which forced their fleas to venture for alternate hosts, including man.

Post-earthquake assessment of Latur and Osmanabad districts revealed an increased vulnerability to vector-borne diseases, including ectoparasite–rodent borne infections in the area⁸. Temporarily vacant houses are also known to produce a sudden abundance of adult fleas upon the return of the tenants and their pets⁹, a situation analogous to what was discernible in village Mamla. Population explosion of a domestic rodent *Mus musculus* resulted in outbreaks of murine typhus among farmers¹⁰. In village Mamla, therefore, a succession of significant ecological events for about an year appears to have led to a flea-transmitted outbreak of a cryptozootic rodent-borne infection in humans, possibly plague.

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Observations on urban ecology of Surat and bubonic plague transmission in the city

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Observations were made on the urban ecology of Surat city to understand the ecological support system conducive for wild rodent epizootic and its spill-over to initiate human bubonic plague outbreak. The unprecedented flood situation prior to the Surat outbreak was also appraised from the standpoint of plague dynamics. No obvious evidence could be gathered relating to the ecological support system and ecological linkages between rodents from urban and feral habitats which could have led to the spill-over of sylvatic rodent plague to initiate bubonic plague in humans. Flood conditions in Surat also could not be identified as a factor contributing to an epizootic of plague and subsequent epidemic of human bubonic plague.

DURING September–October 1994, Surat city in Gujarat State witnessed an outbreak of suspected plague (1027 cases) of which 52 died¹. Although Surat lies close to the northern tip of an erstwhile endemic centre along the eastern watersheds of the Western Ghats in Maharashtra State, the city does not figure in the endemic zone of western India². In May 1995, efforts were made to study the ecology of Surat city and human association with rodent habitats. The primary objective of these field observations was to identify factors which may have led to a plague epizootic among rodents with subsequent spill-over to humans leading to an outbreak of bubonic plague.

Extensive observations were made all over the Surat city with regard to housing pattern, population distribution, developmental activities and industries. Rodent burrows were examined at several places both in the town as well as the periphery of the city. Several interviews were conducted with the local population and the municipal health officials on rodent control activities in Surat city.

The urban area of Surat city is spread out in about 140 sq. km, with a population of about 1.5 million. The city is divided into 6 zones – North, South, East, West, Central and South-West (Figure 1). The North zone and part of Central zone are low lying areas. River Tapi passes through the city and separates the West zone

from the rest of Surat city. During the past 2 decades, the city has witnessed rapid expansion of the urban area on its periphery, and there has been gradual encroachment of ruderal sites as well as cultivated fields, for development of housing complexes. The general sanitary and hygienic conditions of these residential colonies were poor.

The Central zone of the city represents old Surat city and has the highest population density, with about 1/3 of the total city's population (0.5 million) residing here. The population in the 6 zones of Surat city (in descending order) is Central zone > South > East > North > West > South-west.

Surat is a highly industrialized city and the industries mainly comprise of diamond processing, metal works and polyester textiles. All industries are located within the city limits. The entire urban waste (solid, liquid as

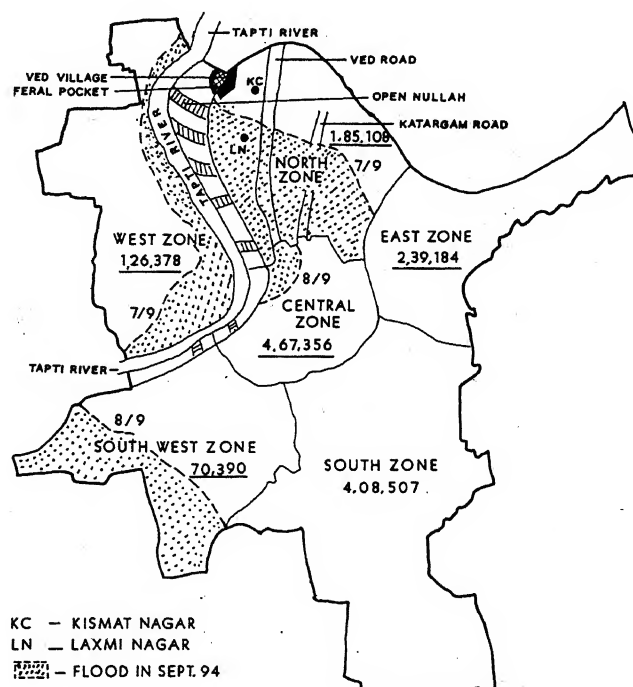


Figure 1. Map of Surat showing city zones and ecological features.

SPECIAL SECTION: THE PLAGUE EPIDEMIC OF 1994

Table 1. Disposal of dead animals by Surat Municipal Corporation

Month/ Year	1992		1993		1994	
	Big animal	Small animal	Big animal	Small animal	Big animal	Small animal
January	91	155	79	180	98	172
February	84	169	63	175	76	162
March	66	183	86	201	106	180
April	69	187	55	193	61	213
May	58	190	58	207	88	201
June	95	205	85	215	102	266
July	91	195	102	198	201	226
August	154	217	83	203	204	861
September	142	214	76	220	355	1127
October	108	177	103	153	277	461
November	120	186	76	171	149	203
December	106	155	76	165	132	179
Total	1104	2233	942	2281	1849	4251

well as industrial) is disposed off through wide open nallahs and, at some places, through closed city drains, into the river Tapti.

The periphery of Surat city has rapidly expanded, particularly in the North, West and South-west zones, with emergence of several slums. The South-west zone has mainly occupied ruderal habitats for urban expansion, but the North and West zones have encroached upon both waste land as well as cultivated land.

The urban area of Surat in the Central zone and its surroundings in the North, East, South zones (Figure 1) do not support habitat diversity congenial for survival of sylvatic rodents. However, the area is conducive for the growth of domestic rodents like *Rattus* species (e.g. *R. rattus*, *R. norvegicus*) and house mouse (*Mus* spp). The land, on both sides of the Tapti river appeared to harbour the field rodent *Bandicota bengalensis*. However, near the bank of Tapti, about 2.5 km away from Laxminagar colony (believed to be the *index case locality* during the September, 1994 outbreak), across a wide agricultural field of Ved village (North zone), a small circumscribed pocket of feral habitat with very a scanty population of wild rodent *Tatera indica* was detected, in dry sandy soil (Figure 1).

During the monsoon period (i.e. June–September) every year, the river water in Surat rises above the danger mark and enters the peripheral areas of the West zone. As a result, the few slum areas situated in this zone along the bank of the river, are adversely affected and the population temporarily shifts elsewhere. In the first week of September 1994, the floods in the river were of unusual magnitude (perhaps the highest in 25 years) and the flood waters entered the east of Tapti, inundating several localities in

North, South-west, Central and East zones of the city (Figure 1). Consequently the entire city waste from nallahs and drains, reverted back into the city due to pressure of water from the swelling river and remained *stagnant in the city for 5 days* (7–11 September 1994) and after the flood water receded, a large number of animals were found dead in the city. A comparison of monthly animal mortality (for 3 years) in Surat as recorded by the Surat Municipal Corporation is given in Table 1.

From an ecological standpoint, the circumscribed, small feral pocket away from human population is not supportive of initiating an epizootic of plague in *T. indica* which may spill-over, to cross the 2.5 km distance interspersed with cultivated land, and enter domestic biotope in Laxminagar colony. The absence of adequate ecological linkages between diverse habitats, essential for the dynamics of plague transmission, does not support the outbreak of bubonic plague in Surat city with its origin in feral habitats. Characteristic biotope associations are a pre-requisite to establish ecological linkages to support spill-over of wild rodent infection to humans^{3,4}. However, if the urban rodent population was enzootic for plague infection, there should have been bubonic plague cases reported even in the preceding years. The floods in September 1994 were of such high magnitude that they must have left many domestic rodents in the city dead along with their flea ectoparasites. The flood waters were highly polluted (containing human, animal and industrial waste), even large animals (cattle, buffalo, etc.) died in large numbers (Table 1). The number of dead animals in September 1994 was 4 and 5 times higher than in September of 1992 and 1993. These animal deaths can be attributed, both to drowning and chemical/organic pollution of flood waters.

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Epilogue – What next?

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The good news is, accepting the recommendation of TAC, the Government of India (vide their order no. T-21011/8/94-PH (Pt. 1) dated 24 April 1996) established a National Apical Advisory Committee (NAAC) for National Disease Surveillance and Response System. The Committee would have the responsibility of playing an advisory role in the establishment of a National Disease Surveillance and Response System.

The terms of reference of the Committee would be as follows:

1. The Committee would give advice and suggest mechanisms for setting up of a National Disease Surveillance and Response System.
2. The Committee would review the progress periodically regarding implementation of the National Disease Surveillance Programme.
3. The Committee would examine the epidemic preparedness of health care providers.
4. The Committee would assist and advise in the development of an early warning signal mechanism.
5. The Committee would review various programmes, guidelines and mechanisms available for prevention and control of communicable diseases with special reference to new, emerging and re-emerging pathogens and epidemic-prone diseases.

There is growing realization today that as new microbial threats are appearing, well-known illnesses thought to have been controlled are resurging¹⁻³. The Acquired Immuno-deficiency Syndrome (AIDS) is a good example of the former while malaria, kala-azar and plague are notable examples of the latter. Emerging or re-emerging, bacterial resistance to drugs is an increasingly serious problem. Population growth and consequent population movements from the hinterland into urban areas and into forest regions, changes in human behaviour, poverty and overcrowding, changes in ecology and climate, floods

and earthquakes, increased tourism, trading and traveling, the evolution and adaptation of microbes and the inadequacy and breakdown of health infrastructures are some of the factors behind the ever-changing scenario of infectious diseases^{2,4}. As far as plague is concerned, the importance of earthquakes, floods and local changes in ecology affecting microbe-carrying rodents and acting as triggering factors is well brought out in the preceding pages. While the canvas of NAAC's operations has been kept very wide covering all diseases, which is to be welcomed, it is to be hoped that Surveillance and Response Mechanisms for Plague and other Infectious Disease would receive the most urgent attention of NAAC for, after all, plague was the agent provocateur.

The essential task before NAAC is to advise the Government on national capability building related to preparedness, early detection and control of emerging and re-emerging infectious diseases and to establish proper disease reporting systems. Establishing the aetiological diagnosis, understanding the sources and modes of transmission of the infectious agents, identifying high risk groups, investigating antibiotic sensitivity of isolated microorganisms and facilitating the development and production of readily available reagents for diagnostic purposes are some of the elements of capacity building. Clinical samples and microbial isolates need to be referred expeditiously to designated laboratories in the country for further study and, at times, they may be referred to centres abroad and the WHO could be a useful ally in such tasks. While national networks of laboratories and scientists would need to be developed on a disease basis, it is equally important to participate in the establishment of a global disease surveillance and response network. The need for this is amply borne out by the fruitful interaction that took place between Indian scientists and scientists located in the WHO Reference Centres on Plague at CDC, Fort Collins (USA), Paris and Stavropol (Russia) in the plague outbreaks of 1994.

To meet the challenges posed by emerging and re-emerging diseases, a strong research and training effort in laboratory and field work is needed on a continuing basis. A cadre of well-trained microbiologists, epidemiologists, clinical scientists, behavioural scientists, entomologists, mammologists and public health professionals has to be built. The knowledge base generated by the research and training effort would enable the public health specialists to design appropriate control strategies and to provide the needed preventive and therapeutic tools. NAAC would need to work in close collaboration with the national research agencies (ICMR, CSIR, DBT, DST and others) and their laboratories in developing an integrated approach to national disease surveillance and response system and to ensure that the talent available in the country is fully utilized.

In the course of this work, TAC has realized the necessity of setting up appropriate containment facilities for handling hazardous microbes in the country. There is also an urgent need to establish laboratory facilities for collection, storage and maintenance of microbial strains isolated from various infectious disease outbreaks. The importance of this facility was brought home to TAC when it realized that despite many plague outbreaks in India, there are only a few old strains from these years that have survived in any of the national and world collections to compare with, thus severely limiting a consideration of the relationship of the strains isolated in the plague outbreaks of 1994 with pre-existing strains in the country and with strains elsewhere. In any national in-

fectious disease surveillance system, it is of the utmost importance to determine the origin of the infection and its relationship to earlier pre-existing strains of the infectious agents in a given area. The Department of Biotechnology is now coordinating an inter-agency initiative to address this issue.

NAAC's establishment represents a landmark in the story of India's struggle against tropical infectious diseases. Disease surveillance for disease prevention is a responsibility of the State and the resources needed must be found. Giving the Aryabhata lecture at the Indian National Science Academy on 4 October, 1994 (ref. 3), I said: 'Finally, attention was drawn to the need for increasing resources for sustainable health development and to the fact that securing the health of the people is a pre-requisite for maximizing wealth creation. Surat is a supreme illustration of the health rationale of development and of the futility of creating wealth without securing the health of those that toil for wealth creation'.

1. Lederberg, J., Shope, R. E. and Oaks, S. C., *Emerging Infections*, Institute of Medicine, National Academy Press, Washington DC, 1992.
2. *Infectious Diseases - A Global Threat*, Report of the National Science and Technology Council, Committee on International Science, Engineering, and Technology working group on 'Emerging and Re-emerging Infectious Diseases', September 1995, p. 55.
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4. Ramalingaswami, V., *Curr. Sci.*, 1996, 70, 1050-1056.

Book Review

Plagues: Their Origin, History and Future. Christopher Wills
HarperCollins Publishers, 77-85,
Fulham Palace Road, Hammersmith,
London W6 8JB, UK. 1996. 324 pp.
Price: £13.95.

The medical lexicon has usurped the word plague to refer to the disease caused by *Yersinia pestis*, although lay English usage (as in plagues and pestilence) adopts a much wider definition for the term. In his book, Christopher Wills also employs it in this larger context as he describes the toll of human mortality and morbidity exacted by a select list of infectious diseases both in the past and in the present-day world. Needless to say, India and its infamous plague epidemic of 1994 merit considerable attention in his discourse.

Implicit in the world plague is a sense of fear, morbid panic and mass hysteria, sometimes bordering on the unjust and irrational, and Wills does well to describe these reactions in respect of the diseases he chooses to cover: the plague, cholera, typhoid fever, malaria, tuberculosis, syphilis and, of course, AIDS. Strangely, the author excludes smallpox, a scourge comparable to those he covers, from his list. He does so on the ground that this disease no longer exists. But then an analogous argument could be made for syphilis, which has lost its sting, if not its bite, since the advent of the penicillin era.

I found Wills to be a fascinating story-teller. Without being encyclopaedic, he mixes history with science with anecdotal information and personal glimpses to sustain the reader's interest all through. For each disease in the list, Wills recounts (not necessarily in that order) its description in historical records, the discoveries associated with research on the infectious agent, its virulence characteristics and its mode of spread and the circumstances surrounding its epidemic outbreaks. The *Yersinia* plagues of the Justinian and the Black Death, the inconclusive debate on whether or not syphilis in Europe pre-existed Christopher Columbus, the controversy between Ronald Ross and Battista Grassi con-

cerning the discovery of malarial transmission by the *Anopheles* mosquito vector, the first documented experiment in epidemiology involving John Snow and the London Broadstreet water pump, and the saga of 'Typhoid Mary' are all recounted here, and in a manner refreshingly different from other accounts on these episodes that I have read earlier.

The author explains that each of these diseases represents but 'the eyes of the [submerged] hippopotamus' of infections in the affected communities and, moreover, that plagues are not confined to the human species but occur in other animals and plant species as well. He also discusses the interplay between pathogens and hosts in shaping the evolution by natural selection of each other's genetic make-up, and is particularly brilliant when he draws upon the analogy of tropical rainforest diversity in explaining the existence of extensive genetic polymorphisms in populations of both hosts and their pathogens.

A variety of events have to fall into place for an epidemic of vast proportions to occur, and breaking any one of the links in the chain is often sufficient to ensure that it does not propagate. 'It is one of nature's supreme ironies that, during the unusual juxtaposition of circumstances that leads to an outbreak of plague, all the major participants in the drama are sick. This includes the humans, the rats, the fleas, and the very bacteria themselves.' Wills attributes the low mortality associated with the Indian plague epidemic to the instantaneous and widespread use of insecticides and tetracycline. The author refers to these as 'Band-aids' that were successful in the short term but which do not address the underlying reasons that provoked the epidemic in the first instance.

I can fault the author on only one count in this book, which is that he repeatedly falls prey to the trap of anthropomorphic word play in describing the properties of the infectious agents. Witness, for example, 'The plague bacillus... is so fragile that it must be cautious. If it behaves with wild abandon and causes the plague, then most of the time it will die as well.' Or at another place, 'Like the strains of typhoid that

have managed to leave the tropics, the malaria species that have done so are more sophisticated than the one that was left behind. Temperate-zone malarias can conceal themselves... and they seem far more adept at surviving in their mosquito hosts.' To be sure, Wills himself is aware that these properties of the pathogens have not been actively acquired but passively selected in the course of biological evolution, and he uses these statements only to make the treatment of his subject less drab. But in these days of publicity for flesh-eating bacteria and machines that think, his presentation is bound to reinforce the pre-existing prejudice.

In the same vein, he addresses the question of the likelihood that a new disease will arise which is associated with both high mortality and rapid spread, and very reassuringly states: 'I am confident that no terrible disease will appear that slaughters us by the billion. The reason is that we can now respond very quickly to such a visible enemy.' I concur that the scenario of a new plague is extremely improbable, but for a different reason. The environmental niches occupied by humans today are not vastly different from those that existed at various times in the past (on an evolutionary time scale), and should a disease of the dreaded kind be waiting to occur, why has it not done so already? This is just another version of the anthropic principle, familiar to (but not necessarily agreed upon by) all physicists – that the very fact we exist today imposes constraints on the properties of the universe in which we live.

For whom is the book intended? Although it is charmingly written, I think it will still be heavy reading for the layman. But to any student (and I do not use the word in its strict pedagogic sense) of science, in particular of biological science – inclusive of medicine and agriculture – I believe that the book has plenty to offer. Wills educates even as he entertains. As is usual, however, the book is priced out of reach for the average Indian, but all libraries may be encouraged to obtain a copy.

I end this review on a personal note. Wills discusses, in a balanced manner, the controversy surrounding the nature

SPECIAL SECTION: THE PLAGUE EPIDEMIC OF 1994

of the etiologic agent responsible for the Indian epidemic in September–October 1994, and records the views of Jacob John from Vellore that it was not *Yersinia pestis*. On 1 October of that year, my wife and I travelled with our sons, then aged six and two, by train from Hyderabad to New Delhi; and then onward to Ludhiana on 4 October by another train that was on its way from Bombay to Amritsar, and that had indeed passed through Surat enroute. This was the time of the Dussehra school holidays, and yet both trains were running near empty. On a platform at the New Delhi railway station, one saw a set of tables that represented a make-shift plague surveillance unit, and many among the general population were moving around with surgical masks or

handkerchiefs tied across their faces. As Wills notes in his book, almost all international air carriers decided to overfly Delhi during those panic-filled days.

So were we being foolish to risk our lives and those of our children in undertaking this journey? Both of us had been students of Jacob John in Vellore and were certainly influenced by his statements, then widely reported in the press, that the Surat epidemic was not the plague. Now that the official report has proved him wrong, does ours become a foolhardy decision in retrospect? I still do not believe so. It was then already the third week since the start of the epidemic, and at least going by the press reports, the disease was not spreading as rapidly as the pneumonic plague is feared to do. Wills would attribute its

mildness to the large-scale consumption of tetracycline, but he was not there at the time. The antibiotic had simply disappeared from the market even in Hyderabad, where the scare was minimal; the increased consumption could not have occurred in the initial two weeks of the epidemic. My own hypothesis is that either the Indian epidemic was caused by a less virulent strain of *Yersinia pestis*, or the population enjoyed a certain degree of immunity to infection, perhaps because of cross-reacting antibodies. The Surat strains of *Y. pestis* demand further study.

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2. Constantine, G., in *Biology of Bats* (ed. Wimsatt, W. A), Academic Press, New York, 1970, vol 1, pp. 319-322.

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COVER. Visualization of the JGOFS theme. The black line shows the area of investigation in the Arabian Sea. The cover also shows ORV *Sagar Kanya*, the research vessel used for measurements. The phytoplankton depicted are the main agents in carbon fixation in the oceans and CTD (Conductivity, Temperature, Depth) system was used for measuring various parameters. See special section.

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In this issue

TRNOESY

Nuclear Magnetic Resonance (NMR) has grown, during the last two decades, into an extremely powerful technique for studying structure, dynamics and interactions of biological macromolecules in aqueous solutions; thanks to the discovery of two-dimensional NMR in the early seventies, which allowed display of interactions between nuclei in a molecule onto a plane. Among the variety of 2D NMR techniques which have been developed over the years, nuclear Overhauser effect (nOe) based NOESY occupies a central place. It displays correlations between protons which are closeby in space and the intensities of the correlations are roughly proportional to the inverse sixth powers of the respective interproton distances. Quantitative interpretation of these pairwise correlations forms the basis of the accepted protocols for molecular structure determination today. Proteins and nucleic acid segments in the molecular weight range of 5–10 kDa are being investigated fairly routinely using these protocols. For larger systems the procedures are still evolving and are more demanding in terms of spectrometer and sample requirements.

In addition to the knowledge of the three-dimensional structures of the individual molecules, a similar knowledge about their complexes is crucial for understanding many of the biological functions. The difficulties and the complexities increase manifold, the moment one goes from isolated molecules to molecular complexes. Moreover, a protein molecule of functional interest may sometimes be too large for a detailed structure characterization with the presently established protocols. In such situations, 'transferred nOe' provides an elegant part solution. When the ligand for a protein macromolecule is small

and is exchanging rapidly between its free and bound forms, the nOe correlations observed reflect on the interactions in the bound form of the ligand, even though this may be a minor component in the solution. A NOESY spectrum displaying such 'transferred nOe' correlations is referred to as a TRNOESY spectrum. This technique has been successfully used by Maity and Jarori (page 906) to determine the conformation of ATP-Mg bound to a specific site on a large protein, namely, bovine serum albumin (BSA). They observe that, on protein binding, the sugar ring in the ATP molecule undergoes a substantial geometrical distortion. This is a reflection on the extent to which the three-dimensional structure of a protein can, in general, influence the conformation of a ligand bound to it by non-covalent forces.

R. V. Hosur

General relativity

This issue carries a Research Account by C. V. Vishveshwara entitled 'On the black hole trail...: A personal journey' (page 824). This was delivered at the IAGRG (Indian Association for General Relativity and Gravitation) annual meeting as the Vaidya-Raychaudhuri Endowment Award Lecture. As is well known in the community, Prof. C. V. Vishveshwara's Ph D thesis made a seminal contribution to the physics of black holes by settling the question of the stability of the Schwarzschild (i.e. nonrotating) black hole against small perturbations. This article provides some fascinating background to that work in terms of circumstances and personalities involved in General Relativity in the late sixties and early seventies. It then goes on to describe later work by the author and his colleagues in areas such as

quasinormal modes, gyroscopic precession, ultracompact objects, etc. and place it in the overall context of other developments.

R. Nityananda

Understanding the oceans

Biology, geology, oceanography and chemistry intersect in the special section (page 801–905) on the Joint Global Ocean Flux Study (JGOFS), which is a core project of the International Geosphere Biosphere Programme. The focus of the articles in this issue is the Arabian Sea which exhibits remarkable seasonal variations in biological productivity. Physical processes, most importantly monsoon-driven circulation, modulate biological events, which in turn affect CO₂ air-sea exchange fluxes and carbon cycling in the ocean. Understanding these events requires a marvellous interplay of physical, biological and geochemical studies, many of which are detailed in the special section. Painstaking observations in this area hold out the promise of future development of predictive models which may allow global scale predictions of the 'response of oceanic biogeochemical processes to anthropogenic perturbations, in particular those related to climate change'.

Lipid clusters

Studying cluster formations in diverse systems is a fashionable activity nowadays. Armed with ideas of fractal dimensions and scaling laws, Lahiri *et al.* (page 915) investigate the aggregation of phospholipid vesicles using straightforward microscopic methods. The demonstration

that addition of the cytoskeletal protein, spectrin, stabilizes cluster formation is of relevance in future studies of vesicular assembly.

Folklore and contraception

The Adivasi tribes in Bihar reportedly use the Banjauri plant (*Vicoa indica*) as a contraceptive, with a powdered

concoction being consumed by women. Following up on this practice, Rao *et al.* demonstrate the antifertility effect of dried Banjauri powder on female bonnet monkeys (page 918). The claims of folklore are investigated by modern scientific methods, providing a promising lead for future development of an active principle as an oral contraceptive. Three pure sesquiterpene lactones from this plant, vicolides A, B, C have been

characterized many years ago, with vicolide B having antifertility activity in rats. Whether new, as yet uncharacterized molecules are responsible for the observed effects and their mode of action should provide fruitful avenues for investigations in future.

P. Balaram

Current Science

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CURRENT SCIENCE

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CORRESPONDENCE

Need to integrate science communication initiatives

Creating public understanding of science in an era when science and technology are permeating every fabric of society needs no emphasis. Despite some encouraging trends in recent years¹, various ongoing science communication initiatives and programmes at the national level need to be integrated under a single accountable authority to avoid duplication of efforts by multiple government agencies.

At the national level, for instance, the renaming and rededication of the New Delhi-based Publications & Information Directorate of the Council of Scientific & Industrial Research (CSIR) as the National Institute of Science Communication (NISCOM) on 25 September 1996 assumes a lot of significance². Interestingly, within the CSIR family, two more New Delhi-based institutes, viz. the Indian National Scientific Documentation Centre and National Institute of Science, Technology & Development Studies are also involved directly or indirectly in science communication.

On the other hand, the Union Ministry of Science & Technology has the popular science wing – the National Council for Science & Technology Communication

(NCSTC). In the recent past, two more outfits have emerged: the NCSTC-Network (a government-cum-voluntary-cum-nongovernmental body) and an autonomous organization, the *Vigyan Prasara*. Interestingly, one individual heads these three bodies. The NISSAT – National Information System for Science & Technology – also falls under the Science & Technology Ministry. Needless to say, the main objective of these set ups in one way or the other is science communication.

Specifically, for health education, the Union Ministry of Health & Family Welfare has a full-fledged institute – the Central Health Education Bureau which is located in New Delhi. And for environment awareness, the Union Ministry of Environment & Forests supports two centres of excellence. These are the Centre for Environment Education, Ahmedabad and the Madras-based CPR Environment Education Centre. The Delhi-based Defence Scientific Information & Documentation Centre of the Defence Research & Development Organization and the Ahmedabad-based Development and Educational Communication Unit of the Indian Space Research Organization are

also major players. Similarly the National Centre for Science Information (established in Indian Institute of Science, Bangalore in 1984) has been active in accessing and disseminating results of worldwide scientific and technological research to the faculty and researchers in universities and other research institutes in the country. Added to all this, the other major S&T agencies have their own Publication(s) and Information Division/Directorate. Even a large number of professional bodies such as the Society for Information Science, Indian Science Writers' Association, Health Media Centre – India and Energy Environment Group are painting a positive picture of science communication.

With this backdrop, there is an urgent need to integrate science communication initiatives.

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Light and the future

Since global environmental and energy problems have reached an alarming situation, 21st century will be the 'age of

light' – particularly a period of exploitation of solar energy. Use of solar energy to combat pollution, to generate chemical energy (by water splitting) and to generate electricity (solar cells) has been reported extensively. Recently, in a two-day meeting, scientists from abroad and India assembled in Madurai to discuss current

trends in photophysics and photochemistry and solar energy conversion.*

As buckyballs are available for the scientists to play, J. P. Mittal (BARC, Mumbai) in his talk highlighted the very unique and interesting photochemistry and photobiology of fullerenes. Using modern laser techniques, the molecular processes

*A Satellite Symposium on Current Trends in Photophysics and Photochemistry was organized in Madurai Kamaraj University, Madurai, during August 5 and 6, 1996.

taking place in fullerenes within picoseconds following the absorption of light energy were studied. Surprisingly, fullerenes were found to be the most efficient compounds to produce singlet oxygen, a form of oxygen possessing high energy. Coupled with this ability and the selective absorption of fullerenes encapsulated in γ -cyclodextrin by cancer cells (compared to normal cells), the feasibility of application of fullerenes in photodynamic therapy, a technique where light energy is used to selectively kill cancer cells, was pointed out.

N. Alonso-Vante (Hahn Meitner Institute, Berlin, Germany) discussed the important role of the surface of semiconductors used in splitting water to H_2 and O_2 , a process where solar energy is harnessed to chemical energy. The results indicate that to tailor semiconductor materials for efficient solar energy conversion, the semiconductor surface is of vital importance besides the photophysics and photochemistry of the semiconductor. Using surface probing techniques such as scanning tunneling microscopy, the role of humidity and the surface structure stability in air were investigated for RuS_2 .

M. Chowdhury (IACS, Calcutta) spoke on the effect of magnetic field on the reaction of transient radicals (unstable intermediate products) produced. The magnetic field can also influence the transient species by bringing them together to form recombined product or to lead them to separate and form new products. The recombination of the photogenerated radical pair has been followed by luminescence from the recombined product (exciplex) and measurement of absorption of the transient radicals by laser flash photolysis. The magnetic field effect (MFE) on exciplex emission varies with the nature of the medium and this change is attributed to the change in the diffusive

motion. The role of polarity and ionic strength of the medium on the magnetic field effect was also highlighted.

Well-known inorganic complexes and organic dyes were incorporated into several synthetic polymers to produce light-absorbing macromolecules. Measurement of the lifetime of the light emission states of these macromolecules was used to map the environment around the light-absorbing antenna molecules. On the basis of these established facts, P. Natarajan (CSMCRI, Bhavnagar) discussed how to devise materials that can mimic photosynthetic biological systems for the production of value-added products from solar energy.

E. Pelizzetti (Univ. of Torino, Italy) focussed on how solar energy could be used to detoxify organic pollutants using the magic chemical titanium dioxide (Figure 1). When semiconductor particulates are irradiated by light, very reactive radical species are produced. Chlorinated organic compounds and some nitrogen compounds which get into the ecosystem by the use of pesticides, coolants, etc. are oxidized by the hydroxyl radicals and mineralized. He also explained how a model waste water treatment plant was in commission in Europe and how the operation of such a plant could be optimized by selecting proper supported photocatalytic semiconductors such as titanium dioxide, suitable light flux and efficient reactor design. For a country like India which has plenty of sunlight throughout the year, such a technique holds a great potential.

H. Tributsch (Hahn Meitner Institute, Berlin) discussed the new concept of proton transport (opposite to the movement of electron) in biomembrane followed by light absorption. Proton exchange with semiconductors such as $ZrSe_2$, TiO_2 , FeS_2 and $InSe$ and its

implications in solar energy conversion was explained. The photophysical processes in bacterial rhodopsin could be explained in terms of proton transfer model based on autocatalytic mechanism.

Photo-induced electron transfer is a key process in biological systems. The distance between molecules involved in the electron transfer is a critical factor for designing molecular electronic devices based on photochemical/photophysical properties. M. Kaneko (Ibaraki Univ., Japan) discussed the use of polymer membranes incorporating photoredox molecules to study electron transfer distances in model systems to understand whether the electron transfer is through chemical bonds or through space or via other mediator molecules.

One of the most promising techniques for solar energy utilization is the design of suitable photoelectrochemical cells. K. Kalyanasundaram (Swiss Federal Institute of Technology, Switzerland) discussed how solar cells can be designed to deliver conversion efficiency of nearly 10%. This efficiency is achieved by judicious choice of materials and appropriate molecular engineering of various components in the solar cell (Figure 2). For maximum light absorption polypyridyl complexes of transition metals were used as photosensitizers. The redox properties of these dyes were tuned to obtain sufficient driving force to convert absorbed light energy to the charge carriers in the semiconductor. Other factors such as solubility and long-term photostability of the sensitizers, good communication between the dyes and the semiconductor surface are also of critical importance to obtain a sustainable technology. Kalyanasundaram exhibited solar cells developed on these principles.

P. Ramamurthy (Univ. of Madras) discussed how radical intermediates formed by light absorption could be used for

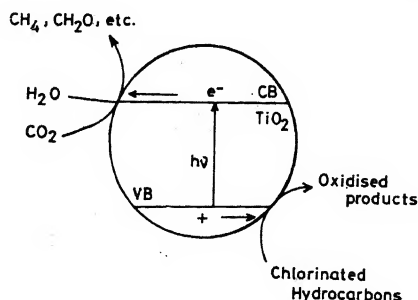


Figure 1. Detoxification of organic pollutants using TiO_2 .

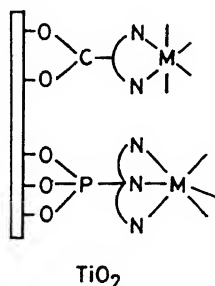
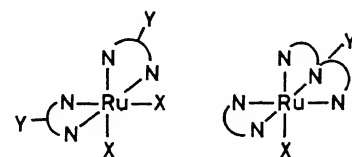


Figure 2. Materials and molecular engineering in photoelectrochemical cells.



production of polymers by taking triphenylpyrylium ion (TPP⁺) as the sensitizer. Using time-resolved light emission studies of the sensitizer, he explained the *in situ* generation of cationic initiators from iodobenzene (PhI). To understand the mechanistic details of the interaction between PhI and triplet state of TPP⁺, electron transfer quenching reaction was studied with a series of electron donors and the theories of electron transfer applied.

Because of their extensive use in tannery, the chromium complexes attract wide interest. T. Ramasami (CLRI, Chennai) explained the photochemistry of cobalt and chromium complexes. He explained how changes in electronic structures, geometries and solvents could influence the photochemistry of these complexes. The role of charge transfer excited states leading to different types of photoredox reactions was highlighted by taking some typical model systems.

S. Mazumdar (TIFR, Mumbai) illustrated the use of picosecond time-resolved fluorescence and anisotropy decay of natural porphyrins and their zinc complexes in anionic, cationic and neutral

micelles. The spectral data are consistent with the model, suggesting that the fluorescence depolarization occurs by both rotational and translational diffusion of the porphyrin inside the micelle along with the tumbling motion of the micelle as a whole.

From the surface photovoltage (SPV) studies, the reversal of the photocurrent in a liquid junction solar cell from n- to p-type characteristics was reported. This reversal is explained by preferential trapping of photoelectrons which supports the widely-held belief that charge transfer across a semiconductor-liquid interface occurs via trapped carriers. Based on SPV studies, the effect of humidity and film thickness on the ability of intrinsic semiconductors to generate photocurrent, the effect of etching on surface change and charge carrier sites have been investigated in the Weizmann Institute, Israel. G. Hodes gave a graphic account of the role of surface states in the photoelectrochemical properties of nanocrystalline CdSe films.

G. Prabhakara Rao (CECRI, Karaikudi) discussed the impact of chemically modified electrodes on many electrochemical

phenomena involved in photoelectrochemical cells. When potassium hexacyanoferrate was used as the chemical modifier, a large enhancement in photocurrent was noticed. However, dark current also increased owing to the formation of reactive intermediates, thereby restricting long-term stability of these electrodes.

P. Velusamy (Gifu Univ., Japan) elaborated the technology of obtaining thin lead oxide films and their interesting semiconducting properties. The photoelectrochemical and spectral responses and solar energy conversion efficiency of films grown using different electrolytes were analysed in the light of surface morphologies of the films.

The diversified topics covered in the meeting have been successful in channeling thoughts and experiments to harness and utilize solar energy and understand several photochemical and photobiological processes.

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RESEARCH NEWS

Anomalous diamonds

Some recent reports of diamonds in rocks of crustal origin have added to the plethora of diamond finds outside the long established kimberlite (diatremes) source. These have shaken conventional notions that diamonds are solely mantle-derived, formed under high pressure and temperature (40 kilobars and 950°C), conditions not available in the crustal environment. Porous aggregates of micrometer-sized diamond crystals—carbonados—are examples¹ of non-kimberlite source showing attributes for a crustal origin. They are considered to be products of either transformed carbon subducted into the mantle or generated during impact metamorphism of carbon in rocks. Irradiation of organic carbon by fission fragments is found capable of inducing structural re-arrangement of carbon to diamond during the

radioactive decay of uranium in uraniumiferous sediments. Such nanometer-sized diamonds have been reported² from Precambrian carburanium—a fine-grained coal-like assemblage containing hydrous, carbonaceous material (with 5% uranium oxide)—occurring as brittle hygroscopic oval inclusions in pegmatite veins from North Karelia, Russia. These 40 nm sized diamonds were retrieved from the acid leached residues of the rock and their identity established by high resolution transmission electron microscopy. The find proves that 'carbonaceous material, catastrophically disrupted by energetic particles, can crystallize as diamond'², a process that will shortly be verified experimentally in a linear accelerator.

Diamonds of extra-terrestrial source^{3,4}, generated in the inter-stellar space, have

been found in iron meteorites and urelites and they are believed to have formed by vapour condensation. Clay beds from the impact crater site along the Cretaceous-Tertiary boundary (K-T boundary)⁵ were found to contain minute crystals of diamond. They are not considered extra-terrestrial but are supposed to be meteorite-impact induced as they show chemical and isotopic constituents typical of earthly materials. Another example of impact-related diamond has been reported⁶ from the 15 million-year-old Ries Crater in Germany. This is considered to have formed 'by chemical vapour deposition from the ejected plume of the impact crater' when carbon-bearing rocks vapourized during the impact.

Among the few other recent non-kimberlite diamond finds, is one from meta-

morphic rocks of southwest Norway carrying crystals, 20–80 μm in size⁷, generated when carbon-bearing sedimentary rocks were carried down to mantle depths temporarily during plate collision (with Scandinavia) and subsequently brought up to crustal levels. Some geologists doubt this, as they consider that crustal rocks are too buoyant to be carried down to mantle depths, and according to Stephen E. Haggerty, the well-known authority on diamonds, they were perhaps produced by a process akin to current industrial practice of making thin films of diamonds by chemical vapour deposition (CVD technique).

Even though the rather varied modes of occurrence of diamonds in nature have jolted conventional ideas about their genesis and exclusive association with kimberlites, the latter rock types still appear to be sole repositories for commercially viable crystals. Their ubiquitous existence and ability to survive over long geological time spans is of considerable significance as they can provide valuable information about impact structures and give 'access to events that occurred (and possibly carbon reservoirs that existed) in the early history of Earth when bombardment by meteorites was at its most intense'.

million years, and hence, oldest Ediacaran fossil). These organisms were globally distributed and belong to late Precambrian period, almost after 90% of geologic time had lapsed, during which life on Earth was represented by bacteria, algae and unicellular organisms. These soon gave way to larger and complex forms which were collectively called Ediacaran fossils and the period prior to Cambrian times were dominated by these forms so much that it came to be known as 'Ediacaran' marking the early Phanerozoic (greek for visible plus animal life)⁵ characterized by these oldest known multicellular life (metazoans). Martin Glaessner, 'the father of modern micropalaeontology', has provided detailed descriptions of these fossils, barring a few of which, most of them became scarce by about 500 million years. The fossils exhibited three dimensional preservation as sediment-filled moulds (i.e. body shape infilled with sediments without any trace of anatomical features) and without substantial flattening. Though palaeontologists initially classified them as animals, later researchers had expressed views that they may be plants or giant single-celled organisms or even as an unsuccessful evolutionary experiment completely different from known life on

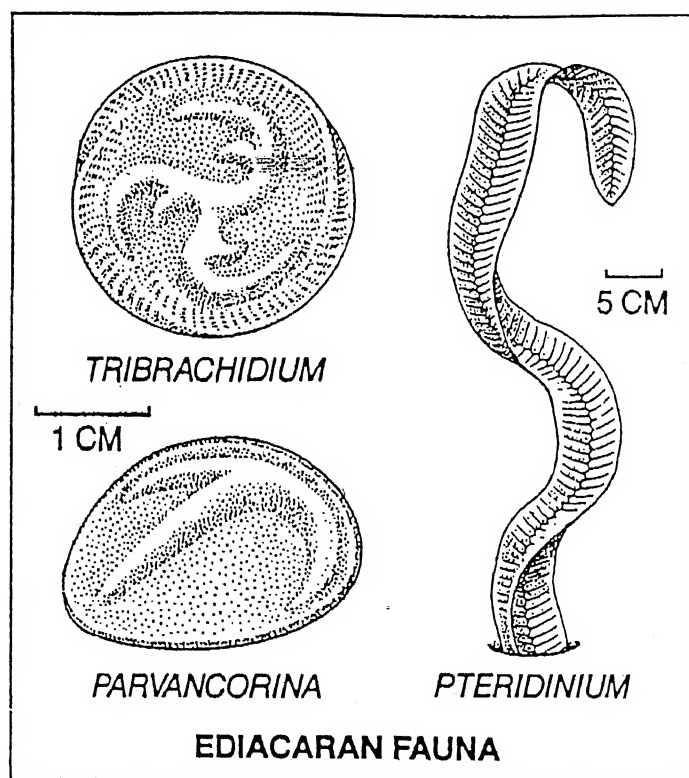
Earth. In fact they were considered enigmatic for quite a long time.

However, recently a novel idea has come up that the Ediacaran fossils are remains of large lichens which had widespread development in the Precambrian and they were believed to be living symbiotically with algae and bacteria of those times^{8,9}. The animal-jellyfish connection suspected by the earlier discoverers was dismissed by experimentally proving that soft-bodied animals like jellyfish cannot leave any fossil impression as the weight of rocks piling over would have squeezed them much flatter and hence the Ediacaran fossils must have had structural strength and rigidity of the large plants having chitin. Like plants, they produced their own food and had microscopic tube-like structures similar to modern lichen filaments. These views have been discounted by other palaeontologists some of whom consider that the author had insufficient data and his conclusions based on structural strengths are flimsy as all Ediacaran fossils are merely impressions in rock and not fossilized remnants; also, their resemblance to sea-anemones (instead of jelly fish as earlier put forward) would be more apt as they had stiffer body. On anatomical grounds too, most of them

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Ediacaran fossils – lichens?

In 1946, Precambrian fossil impressions of simple, soft-bodied organisms believed to be related to jelly-fish were described¹ from Ediacara Hills in Southern Australia and similar fossils were later found in Namibia, Ireland, China, Siberia, New Foundland, British Columbia, N. Mexico and in India^{2–7} (claimed to date 600



A few forms of the Ediacaran fossils.

bore resemblance to 'animals' though some forms like *Tribrachidium heraldicum* with three-fold symmetry, not seen in modern organisms, do not fit into any category. The biological enigma about these Ediacaran fossils still persists. Perhaps they belong to diverse groups – some resembling corals, some the mobile animals, while a few others, acellular; in fact they can be interpreted to represent a whole spectrum of organisms.

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Paucity of preserved old crust

Though Earth's oldest crust formed more than 4 billion years ago, today researchers are baffled by the paucity of early continental crust. Different models have been put forward by them to explain this. According to one view, oceanic crust gets recycled into the mantle by plate tectonics while the continental crust, being too

buoyant to be drawn back into the mantle, had persisted and got weathered soon. Another view assumes that continental crust grew very gradually and their scarcity is due to their smaller size during Earth's early times and perhaps the creation of new crust and destruction of old ones had balanced since then.

An answer to the riddle about the rarity of early crust has come out of recent studies by Bowring and Housh¹ who through Sm–Nd isotopic studies have proved that indeed large segments of continental crusts have been recycled into the mantle during 4 billion years of Earth's evolution. They have used the progressive variation in the Sm–Nd ratios in the mantle reservoir through time as the basis for distinguishing older from later or 'juvenile' crust. The variation in the Sm–Nd ratio is brought about during the mantle differentiation which results in the formation of a buoyant crust. In this process, the newly formed crust gets preferentially enriched in Nd causing depletion of this element in the mantle reservoir through time. Thus the older of the early continental crusts will have ratios closer to the initial Sm–Nd ratios of the mantle and the younger ones, the products of mantle reservoir with depleted Nd, will correspondingly reflect the later Sm–Nd ratios. The analysis of fragments of early Archaen rocks indicate that even though large amounts of continental crusts formed early in Earth's history, they were subsequently destroyed and replaced by 'juvenile' rocks.

Direct unambiguous evidence of such recycling of early crust has come up recently in the find² of a diamond from a kimberlite pipe in N. E. Swaziland in Africa having as an inclusion, a crustal mineral – staurolite (an Fe–Al silicate).

This mineral is typically developed in metamorphosed clay-rich sediments and never reported from the mantle. This staurolite was apparently carried down into the mantle by subduction and later became encapsulated by diamond when the latter nucleated around it. The diamond was subsequently brought to the surface through the kimberlite magma. In another recent report³, a large chunk of peridotite in crustal gneiss in Alpe Arami region (Swiss Alps) has been found and this is considered yet another evidence of recycling of continental crust. Here, the large chunk of peridotite is believed to have been picked up by the continental gneiss of this region during its transit deep into the mantle (300 kms or more), when the gneisses were subducted during plate collisions, and subsequently brought back to the surface driven by buoyancy forces accompanying their warm up. In the process the gneisses underwent some metamorphism by the heat and pressure of the mantle. Fresh evidences are steadily coming up, establishing that recycling was an important process during the early history of the Earth and that the 'preserved continental crusts comprise of fragments that escaped recycling'.

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Caution on the use of oxygen electrode-based assay for superoxide dismutase activity in crude preparations

Superoxide dismutases (EC 1.15.1.1) are a family of metalloenzymes that catalyse dismutation of superoxide radicals into oxygen and hydrogen peroxide. Growing literature in this area has signified the importance of superoxide dismutase (SOD) in imparting oxidative stress tolerance to plant and animal systems^{1,2}. This necessitated the development of a number of assay procedures to meet the experimental requirements³.

Flohé and Ötting³ and Beyer and Fridovich⁴ critically reviewed a number of procedures to assay SOD and highlighted some of the associated problems. Working with crude extracts of plant leaves poses a problem of green colour in the reaction medium, which interferes while assaying with any procedure based on spectrophotometry, e.g. NBT (nitro blue tetrazolium) reduction assay. Assay based on oxygen electrode⁵ eliminated some of the problems linked with other assays⁴ and the problem of colour in crude preparations was accounted for. In this assay procedure, riboflavin was photoreduced in the presence of EDTA (ethylene diamine tetra acetic acid, di-sodium salt); reoxidation of reduced flavin generated superoxide radicals utilizing oxygen molecules from the reaction medium. An oxygen electrode was used to monitor this depleted oxygen. In the presence of SOD superoxide radicals are disproportionated to oxygen, leading to the replenishment of the oxygen concentration of the medium. Therefore, an 'apparent inhibi-

tion' of oxygen uptake is recorded by the oxygraph. The degree of inhibition in oxygen uptake was used to calculate SOD activity.

However, the procedure suffers from a major drawback, particularly when applied to crude preparations. As mentioned earlier in a SOD catalysed reaction hydrogen peroxide is also generated. This hydrogen peroxide is likely to be decomposed into oxygen and water by the action of catalases (EC 1.11.1.6) present in the crude extract. Thus depleted oxygen from the reaction medium would be replenished by SOD as well as catalases activity. Consequently, oxygraph will record more inhibition in terms of oxygen uptake, leading to overestimation of SOD activity. Moreover, the procedure⁵ was reported without comparing it with any well-established procedure.

Our effort in the present communication is to study the interference caused by catalases in SOD estimation by oxygen electrode and NBT reduction procedures. Initially, oxygraph-based procedure was tested for its reliability by comparing with NBT reduction procedure, which is a very well accepted method for SOD assay.

Crude extract from leaves (1 g leaf homogenized in 10 ml of 50 mM potassium phosphate buffer, pH 7.8 containing 400 mg PVPP (polyvinylpolypyrrolidone); supernatant was used after centrifugation at 15,000 rpm for 20 min at 4°C) of tea (*Camellia sinensis*), pea (*Pisum sativum*) and barley (*Hordeum vulgare*) and purified

SOD from tea leaves was used to assay by NBT reduction and oxygraph procedures^{4,5}. SOD from crude extract of tea leaves was purified by salting out at 30–60% ammonium sulphate saturation. Precipitate was dissolved in phosphate buffer (pH 7.8, 10 mM), dialysed for 24 h against the same buffer and fractionated on a DEAE (diethylaminoethyl) cellulose column using 50, 200 and 500 mM KCl. The fraction with the highest specific activity for SOD (purified SOD), as determined by NBT reduction procedure, was used for our purpose. The purified enzyme was size-fractionated through a TSK column on HPLC (Data System 450, Kontron Instruments, Switzerland) using phosphate buffer (pH 7.8, 10 mM) at a flow rate of 3.0 ml/min and a major peak with highest SOD activity (extra purified enzyme) was used for experiments involving addition of external catalase (Sigma Chemicals; cat# C 2001).

Purified SOD was assayed along a temperature range of 15–35°C. Both the procedures showed maximum activity for SOD at 30°C with a very high correlation ($r=0.824$, data not shown). The two procedures responded similarly to different SOD concentrations ($r=0.982$, data not shown) along a large range of protein concentration. There was a shift in pH optima for SOD by the two procedures. NBT reduction method displayed maximum SOD activity at pH 7.8 whereas, oxygen electrode procedure revealed the best results at pH 7.4 (data not shown).

Table 1. Determination of SOD activity in crude extract and in purified enzyme by NBT reduction and oxygen electrode procedures^a

Enzyme preparation	SOD activity as per cent inhibition					
	NBT reduction procedure ^b			Oxygen electrode procedure ^c		
	A	B	C	A	B	C
Tea ^d	20.28 ± 4.80	19.48 ± 4.20	21.00 ± 3.65	48.35 ± 0.48	47.96 ± 0.40	61.56 ± 0.18
Pea ^d	22.24 ± 1.90	23.75 ± 1.60	22.86 ± 1.95	70.18 ± 0.87	71.62 ± 1.11	88.64 ± 1.21
Barley ^d	43.21 ± 4.90	44.54 ± 2.30	43.93 ± 5.00	73.00 ± 0.35	72.41 ± 0.53	90.35 ± 0.49
Purified enzyme	46.29 ± 0.66	45.95 ± 0.51	46.09 ± 0.19	29.50 ± 0.15	45.38 ± 1.60	70.77 ± 0.20

^a Reactions were carried out at 30°C; incident radiation, 1000 $\mu\text{E m}^{-2} \text{s}^{-1}$; separate blanks (with boiled enzyme) were prepared for each enzyme preparation.

^b Reaction medium: 0.05 M potassium phosphate buffer (pH 7.8) containing 5.7×10^{-5} M NBT, 9.9×10^{-3} M methionine, 1.17×10^{-6} M riboflavin and $2.5 \times 10^{-2}\%$ (w/v) Triton X-100 in a total volume of 3 ml.

^c Reaction medium: 5×10^{-5} M riboflavin, 1.5×10^{-4} EDTA and 0.5 μM KCl in a total volume of 3 ml of 0.05 M potassium phosphate buffer (pH 7.4).

^d Leaf material was used to prepare crude extract.

A = no catalases or hydrogen peroxide was included during assay; B = catalase (3 U; Sigma Chemicals; cat# C 2001) was included in the reaction medium; C = hydrogen peroxide (75 nmole) and catalase (3 U; Sigma Chemicals; cat# C 2001) added in the reaction medium. \pm represents S.E. values for three separate determinations.

The effect of sodium cyanide on inhibiting SOD activity was well recorded by the two procedures.

Although the correlation between the two procedures was very high, NBT reduction procedure always recorded more inhibition than oxygen electrode-based procedure when purified enzyme was used (Table 1). Such observed variations in enzyme activity by the two different procedures are not unusual. In fact, Beyer and Fridovich⁴ reviewed a large number of procedures for SOD assay and realized a vast difference in the amount of SOD required for 50% inhibition (0.004–0.73 µg SOD/ml) and pH optima (0.2–10.2) for various assay systems. Our results have shown that the amount of protein required for 50% inhibition by oxygen electrode procedure is about 1.5 times higher than that required for NBT reduction test.

While performing assay with catalases free, extra purified SOD enzyme, it was interesting to note that the addition of 3 U catalase (Sigma Chemicals; cat# C 2001) in the reaction medium showed a further inhibition in the rate of oxygen uptake by oxygraph procedure, but had no effect on NBT reduction (Table 1). Thus, the added catalase catalysed the decomposition of hydrogen peroxide into oxygen and water and the oxygraph recorded this change. Since NBT reacts directly with the superoxide radical and the oxygen molecules *per se* have no effect on it, hence no change was detectable by the NBT reduction method. Hydrogen peroxide in the reaction medium will be generated not only by the action of SOD, but the possibility also exists that self-dismutation of superoxide radicals would generate hydrogen peroxide⁶, leading to further release of oxygen by the action of catalases.

When SOD was assayed in crude extracts of tea, pea and barley by the two procedures, inhibition recorded was lower by 58.0, 68.31 and 40.8%, respectively in the NBT reduction method compared to oxygraph-based assay procedures. Our estimations showed very high catalases activity in the crude extracts. This probably explains a higher 'apparent inhibition' recorded by the oxygraph, as discussed earlier. Addition of external catalase in crude extracts had no effect on SOD estimations by the two procedures. Probably, catalases activity in the crude extracts was already present in optimum quantity.

In a separate experiment (Table 1), SOD activity by the two procedures was

assayed in crude extract as well as in extra purified enzyme in the presence of externally added hydrogen peroxide (75 nmole) and purified catalase (3 U; Sigma Chemicals; cat# C 2001). The NBT reduction procedure did not show any difference in SOD activity, whereas oxygraph procedure showed further inhibition that was attributed to the externally added hydrogen peroxide. Inhibition thus produced was very much pronounced in case of purified enzyme (139.8% more inhibition) compared to that observed with the crude extract (23–27% more inhibition). While it justifies our hypothesis further, the results with crude extract point out that apart from catalases other hydrogen peroxide scavenging systems, e.g. peroxidases, which would not release any oxygen molecule during catalysis, might also play a role. However, their contribution will depend on the availability of the substrate(s) in the crude extract. Catalatic or peroxidatic activity largely depends upon leaf age, environmental factors and so on. Hence, results obtained with the crude preparations using oxygraph may be misleading.

Some of the known inhibitors of catalases like salicylic acid⁷ or aminotriazoles⁸ inhibit catalases only partially, while cyanide inhibits catalases as well as copper and zinc containing superoxide dismutase⁹. It was not possible to inhibit either of the enzymes completely to show the role of catalases in overestimation of SOD activity. Therefore, additive rather than the subtractive approach was followed to solve the problem.

While the oxygen electrode-based assay procedure appears to be simple, it does not involve any expensive chemical like NBT and is ideal for coloured preparations, the presence of catalases and other interfering agents, e.g. peroxidases makes it impractical to work with crude preparations. However, the method could be used with highly purified SOD preparations.

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D. Bhatnagar's response:

The determination of superoxide dismutase (SOD) by oxygen electrode (*Curr. Sci.*, 1995, **68**, 960–962) has not been presented for crude preparation and can be used, only with purified enzyme. The crude homogenates cannot be used in this method due to high amounts of peroxidase and catalase and other proteins which should be removed. However, when purified enzyme was used, addition of low concentration of cyanide (0.5 µM) to the reaction mixture inhibit catalase completely without affecting measurement of SOD activity. Low concentration of cyanide (5–50 µM) has been used to inhibit peroxidase and cytochrome oxidase in crude samples while high concentration of cyanide (1–2 mM) has been employed in the differential cyanide inhibition assay to inhibit CuZn SOD and permit the quantitation of both CuZn SOD and Mn SOD (Iqbal, J. and Whitney, P., *Free Rad. Biol. Med.*, 1991, **10**, 69–77). In crude preparations due to the presence of catalase, decomposition of hydrogen peroxide into oxygen will lead to erroneous determination of SOD.

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Vision 2005: Earth Sciences

The Department of Science and Technology (DST) implements many programmes through which research is promoted in frontier areas of science and technology. The Science and Engineering Research Council (SERC) has played a pivotal role in this regard. Taking note of the global trends, SERC has decided to prepare 'Vision 2005 Document' in various disciplines. The Programme Advisory Committee on Earth Sciences has prepared a 'Vision 2005: Earth Sciences' document based on indepth discussions with a wide cross-section of the scientific community. The document indicates the areas of research in Earth Sciences that need to be given added thrust in future.*

Preamble

Man's inquisitiveness about and his dependence on environment and the processes contributing to its change form the basis of studies in Earth Sciences. Over the years, our understanding of the processes operating in the earth has increased considerably. This has led to a variety of new questions and new avenues of research. The emphasis today is to quantify the earth's endogenic and exogenic processes which control its internal dynamics and shape its surface and fluid envelopes, the sum total contributing to global change. Such a study involves not only the contemporary events and processes, but also those of the past, as what we observe today is a cumulative effect of the past and present processes. More recently, study of the interactions among the various components of the earth systems – lithosphere, hydrosphere, biosphere and atmosphere – has gained considerable importance as these interactions influence global change on various spatial and temporal scales.

Studies in the earth sciences in India are also slowly undergoing major changes. The current emphasis is to substantiate the classical approaches of exploration, description and data gathering with quantitative aspects of data processing, and interpretation of processes and events contributing to the observations. It is also evolving from a subject of individual inquiry to larger programmes involving scientists with complementary expertise and capabilities. Thus, earth science programmes are becoming more multi-institutional/multi-disciplinary, with increasing applications of the concepts and methods of mathematics, physics, chemistry and biology.

A major impetus for this shift comes not only from the need to understand and quantify better the spatial and temporal evolution of the Indian lithosphere but also from the recognition that such knowledge could form the basis for sustainable development of our natural resources. In addition, the recurrence of natural hazards has reinforced the need to learn more about the mechanics of these phenomena and develop predictive modelling capabilities.

The emerging challenges require, on a continual basis, appropriate human resource development in terms of skills and expertise, as well as facilities and infrastructure. The direct interface of the Earth Science Programmes with exploration/exploitation of both renewable and non-renewable natural resources (like energy, groundwater and minerals) calls for a much closer tie-up of its R&D programmes with the industry, user agencies and concerned government departments.

Resume of research activities in identified areas and their impact

India is a large country integrating a variety of geological features and phenomena. These include the Archaean cratons and their accreted mobile belts, the Himalaya – a classic example of continent – continent collision process, the Deccan volcanic province – one of the largest outpourings of continental flood basalts on the earth's surface, Proterozoic cratonic basins (Purana basins) of unique character, well-preserved sections of stratigraphic boundaries, some of the largest rivers of the world – the Ganga and Brahmaputra and a variety of well-preserved archives of past climates and environment. Extensive studies of these geological features have resulted in a number of important contributions in the field of earth sciences. Many of these studies stem from the thrust areas identified in the earlier DST document 'Challenging Areas in Earth and Atmospheric Sciences'.

*Composition of the Programme Advisory Committee on Earth Sciences: Prof. R. S. Sharma (Chairman), Shri D. N. Avasthi, Dr S. Krishnaswami, Prof. S. K. Tandon, Prof. S. K. Shah, Prof. C. Leelanandam, Prof. S. C. Sarkar, Dr M. Ramakrishnan, Prof. V. Subramanyan, Dr V. P. Dimri, Prof. S. Mitra, Prof. A. K. Jain, Dr K. R. Gupta (Convener) and inputs provided by Dr U. Raval, Dr S. K. Gupta, Prof. B. B. S. Singal and Dr R. K. Midha.

Future research directions: Objectives and research areas

Considering the available results of the completed and on-going researches, relevant panel reports and existing scientific and social environments, the task before us is to identify areas which hold promise for creative excellence and for the well-being of the society. It is expected that such areas would have a high likelihood of significantly enhancing our basic understanding of the geological processes/events and would yield results of general interest and application. This is indeed a difficult task even in the best of situations, because with the progress of research, new questions continuously arise which could drastically change the directions of enquiry. Nonetheless, we are listing below a few themes/areas for pursuing future research activities to enlarge our understanding of the earth processes. Other factors which have been considered in arriving at the list include: (i) the expertise of scientists, (ii) technology, manpower and financial support which are available at present and which may become available in due course, and (iii) the current international research trend. Additionally, future strategies in earth sciences must take into account basic and applied aspects in order to be able to cater to the ever-increasing human requirements.

While indicating the future directions, all areas/components of earth science were not considered; hence the topics listed below cover only selected disciplines.

Researches in other areas such as oceanography, limnology and atmospheric sciences, are being addressed to by specialized panelists of the DST.

Areas of opportunities

- i) Evolution of the Indian crust
 - 1. Precambrian cratons and mobile belts (including greenstone belts and granulite terrain)
 - 2. Purana Basins
 - 3. Phanerozoic Basins
 - 4. Mesozoic-Tertiary volcanic provinces
 - The Deccan and related traps
 - The Panjal traps
 - The Andaman arc
 - 5. Himalayan Orogenic belt
- ii) Structure and dynamics of lithosphere and mantle
 - 1. Continental crust (thermal structure, thickness and physical nature)
 - 2. Role of fluids (in geological processes)
 - 3. Experimental studies (phase equilibria and physical properties of deep interior)
 - 4. Lithosphere structure and plate dynamics
- iii) Earthquake processes
 - 1. Himalayan seismicity

- 2. Intraplate seismicity
- 3. Neotectonics and Palaeoseismicity
- iv) Reconstruction of palaeoenvironments, palaeoclimates and past global changes
 - 1. Infra-/inter-trappean sedimentary sequences of Deccan province
 - 2. Regolith sedimentology
 - 3. Palaeobiology and environmental shifts
 - 4. Quaternary sedimentation pattern, climate history and global changes
- v) Earth science application for societal needs
 - 1. Geohydrology
 - 2. Mineral resources and fuels
 - 3. Environmental geology and natural hazards
 - 4. Preservation of national heritage

The above-mentioned areas have been briefly discussed in the 'Vision Paper' with regard to some emerging problems under each theme. But we must realize that all geological problems start in the field, and it is strongly recommended that field study be recognized as an inherent and pervasive part of the research/education of earth sciences.

Interactive geoscientific studies

The geological sciences draw on tools and knowledge developed in other scientific disciplines. At the same time, geological research has contributed concepts and techniques to these other disciplines. For example, the structural determinations of high-temperature superconductivity drew heavily on mineralogical principles, and the similarity between the perovskite structure of these superconductors and mineral structure of large parts of the mantle is an example of closed relationship between geoscience and physical sciences. From the curiosity of studying geological materials on submicroscopic domains, the earth scientists also developed or refined several analytical devices (e.g. EPMA, Ion Probe, high P-T equipments) that were then applied in many other fields.

Increased understanding of the earth processes as well as emerging newer concepts and methodologies require interactive research programmes involving geoscientists, physicists, chemists, biologists and mathematicians. For molecular phylogenetics, a palaeontologist has to interact with biologist and organic chemists. Similarly, a petrologist and mineralogist would need a physicist to peep into the intricacy of heat and mass transport problems associated with matter in subcrustal depths. Even within earth sciences most research works are multidisciplinary about which an emphasis has been given in this document. Interactions seem to be legion, and most frontier research topics relate to more than one theme. Again, present day computational capabilities have revolutionized the

handling of vast amounts of data generated in earth science research. We now require to develop quantitative models for a number of earth processes. Even such traditional disciplines as mapping and palaeontology are becoming increasingly quantitative with the advent of digital analysis and computerized data base. The main emphasis of this theme is to recognize research problems in geosciences which need to be seen from different angles since solid earth of a geoscientist is in a way a science of solid state physics. To account for several earth science phenomena we also need to develop theoretical/mathematical models. Compilation of geological history and study of modern processes and their rates would allow mathematical modelling. For example, modelling is required for geochemical cycling, which brings together results from studies of various aspects, including mantle evolution, global tectonics, rock-water interactions, organic evolution and palaeoclimatology.

Numerical computer simulation is needed to develop, rather more rigorously, for a number of earth processes involving inputs from physical chemistry, statistics and other fields. We need accurate projection of the data-based interpretation for prediction of the natural processes. Even chaotic systems are subjected to statistical prediction. For example, the quantitative treatment of isotope exchange between rock minerals and fluids needs intensive integrated research in fluid-rock interaction and fluid flow within the crust. Many research problems of integrated nature can be conceived and formulated, but only a few are mentioned below.

In igneous petrology where study of silicate melts is made, geochemistry is definitely an essential research component. But to advance our understanding of many related processes, such as element partitioning between melt and crystals and crystallization sequence of minerals, a full knowledge of silicate melt structure is necessary. For this we need interaction with physicists and chemists. To study viscosity behaviour of melt, solubility of water in the silicate melts and structural nature of the melts, we need Raman Spectroscopy and polymer chemistry and related fields. In addition, spectra of ferrous/ferric iron requires knowledge of Mössbauer spectroscopy and crystal structure. Furthermore, to elucidate the petrogenesis of diversified igneous rocks, Rayleigh fractionation equation are used for elemental data so that vector calculations can be made for representing the composition of the derived liquids (resulting from the removal of given phases) and of cumulates (resulting from crystallization of model liquid). Clearly, we need inputs from experimental studies of crystal/liquid and element partitioning between them.

The lithospheric evolution of the Indian region forms an important interactive research area that would involve seismological, heat flow, magnetic, gravity, electrical, isotopic and geochemical studies of specified seg-

ments/transects in India. The studies may be carried out in stages as a multidisciplinary research project.

A long-term approach in metamorphic petrology is to outline P-T-t paths which indicate dynamic time-dependent character of metamorphism for a given crustal segment of overthrust belt. The geothermobarometry, based on thermodynamic principles, when applied to zoned minerals or to incompletely reacted mineral assemblages would help to define the paths of pressure/and temperature variation followed by the individual rocks. Chemical data on the zonation of minerals would additionally provide a wealth of information on the thermal processes that took place during metamorphism of rocks and growth of minerals. This shift of metamorphic petrology from a static mode (aimed at working-out the mechanical and thermal processes involved in metamorphism) needs numerical modelling. In this effort, the thermal response of the rocks to tectonism can be determined by computer modelling of the transient temperature distribution in a rock mass of specified physical properties, assuming certain boundary conditions. This forward approach complemented by petrological observations would unravel details of thermo-tectonic evolutionary history.

Manpower development and infra-structural facilities

The successful implementation of research programmes in any field requires personnel in various categories, appropriate infra-structural facilities such as equipments, current books and journals. India has a wealth of experienced earth scientists, but many of them are trained primarily in the classical approaches and are very specialized. The need today is more broad-based and earth scientists with good background in basic sciences, particularly mathematics and physics and chemistry, are required to carry out many of the interdisciplinary programmes and develop capabilities for modelling the results. In this context, there is a need not only to overhaul the current compartmentalized education system in earth sciences in the universities, but also to encourage scientists from basic sciences to immigrate to various areas of earth sciences. The earlier PAC had prepared a detailed document on Earth Sciences Education in India (*Current Science*, 1994, 67, 74-77).

To ensure front line scientific research in earth sciences, a continuous series of training programmes by way of workshops, summer schools, advanced short courses in selected topics, are required to be encouraged. Contact programmes need to be initiated particularly in institutions where infrastructural and instrumental facilities are available such as the WIHG, NGRI, PRL and IIT's. Interdisciplinary teams must be motivated to prepare instructional materials for dissemination. Refresher

- courses in modern trends in earth sciences with basics in physics, chemistry, mathematics and computer applications, mostly of remedial nature, should be formulated and distributed to various institutions/universities largely through video-lectures and correspondence materials.

Research in earth sciences, or for that matter any other disciplines, is considerably influenced by current awareness which in turn depends on the availability of a wide range of journals – both basic and applied. Indian universities in the recent past are faced with a major financial crisis, particularly in respect of library grants. The situation requires immediate redressal by way of long and short term measures. As a short-term strategy, there is an immediate need for five to six regional units equipped with computer database, such as GEOREF together with the required infrastructure for the dissemination of library materials to other users. Earth science data rely heavily on precise measurements. Collection of these data, particularly those dealing with geochronology, isotope systematics, chemical composition, requires sophisticated instruments. This is an area where most of the Indian universities and institutes lag behind the international scene. Consequently, we need to strengthen and augment the instrumentation facilities so that the country's research efforts can be maintained at an internationally competitive level. The approaches for strengthening and updating the instrumentation

facilities are discussed in detail in a separate document prepared by the PAC on this topic.

Conclusion

The potential and promise of research in earth sciences in India, both in basic and applied areas, are vast. The areas delineated in this Vision Paper should be interesting enough to stimulate a scientist in his imagination and to identify a specific problem, suiting his background, resources and infrastructural support that he may muster. The time is now opportune to follow-up some of the most challenging themes intensively by launching national programmes (interactive/multidisciplinary) through appropriate linkages between industry, universities, Government laboratories and survey organizations. Through these coordinated efforts and interaction between scientists of related and varied specialization we are expected to generate a more positive research environment whereby scientists would have access to library and instrumental facilities, with their maximum utilization. As a consequence, these would result in excellent research in the country whose spin off would naturally be toward the development of technology and science that would foster country's economic growth and meet societal needs as well as enhance defence, archaeology, dam, irrigation and geotechnical activities.

On the black hole trail . . . : A personal journey*

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Beginning of the trail

It is a joy to give this talk as a tribute to Professors Vaidya and Raichaudhuri, the two father-figures of general relativity in India. If my talk is rather autobiographical in nature, the responsibility rests with Naresh Dadhich and Bala Iyer, respectively the President and the Secretary of the Indian Association for General Relativity and Gravitation, who persuaded me to make it so.

My personal journey along the black hole trail started in the sixties when I was a graduate student of Charles Misner at the University of Maryland. I had transferred from Columbia University in New York specifically to work with him. I had been encouraged to follow this course by Robert Fuller, my mentor at Columbia University and like Misner a former student of John Wheeler. Fuller had remarked, 'If you want to work in general relativity, why not go to one of the best in the field!' Also, that was when I first came to know about Vaidya and Raychaudhuri. Leepo Cheng was doing her master's thesis with Misner on the Vaidya metric. She was appalled by my ignorance when I told her that I did not know who Vaidya was. Later on, we were told that Raychaudhuri was coming as a Visiting Professor. Again, my colleagues were suitably impressed by my ignorance when I confessed that I did not know who this Raychaudhuri was either. I went on not only to take a course on cosmology from him but also to pass it with a little bit of honest cheating. Never did I dream that some day I would be delivering a lecture in honour of these two gentlemen.

Let me come back to black holes. That is not what they were called at that time. Schwarzschild singularity, which is a misnomer. Schwarzschild surface, which is better. The term 'Black Hole' was to be coined later on by John Wheeler. Perhaps, it was because of such an intriguing name that so many people were enticed into working on the physics of the black hole. This is known as the Schicklgruber Effect. Scholars have specu-

lated on how human history might have been different if Schicklgruber had not changed his name. But, he did change his name to Hitler. Misner proposed the following problem for my Ph D thesis. Take two of these entities that are now called black holes. Revolving around each other, they come closer as energy is radiated away in the form of gravitational waves. They coalesce into an ellipsoidal 'Schwarzschild surface' still rotating and radiating. Study the whole process, computing all the characteristics of the emitted gravitational radiation. Fine,

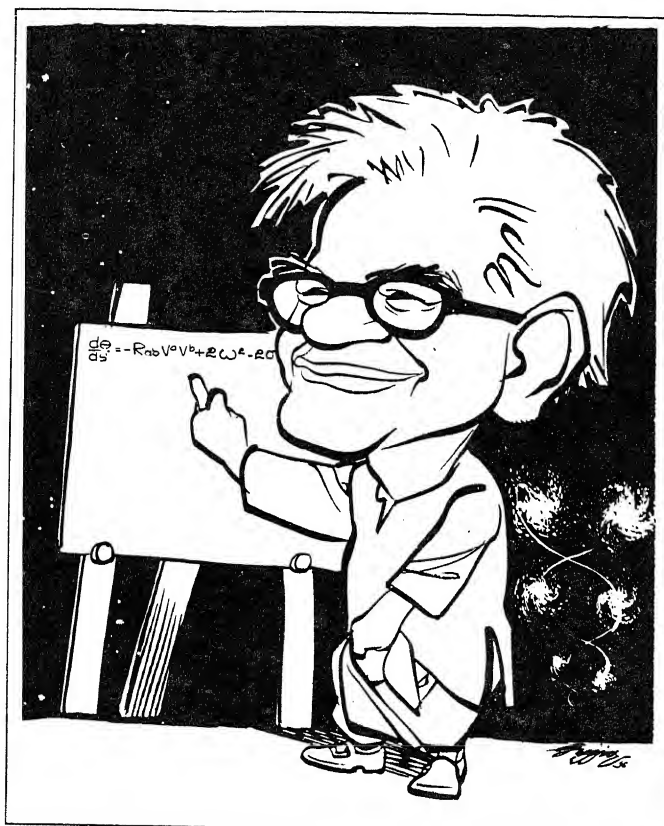


P. C. Vaidya

The Radiant Rider

Discoverer of the well-known Vaidya metric which represents the spacetime of a radiating star. Now in his youthful seventies, Vaidya rides his bike with such a star for the lamp.

*Text of 'Fourth Vaidya-Raychaudhuri Endowment Award Lecture' delivered on 17 February 1996 on the occasion of the XVII meeting of the Indian Association for General Relativity and Gravitation held at the Institute of Mathematical Sciences, Madras.



A. K. Raychaudhuri
The Cosmic Converger

Deriver of the extensively used Raychaudhuri equation which describes the motion of galaxies and shows how they must have emerged from an initial singularity in the past.

I said, thy will be done! No one at the time could have realized the magnitude of this problem. Had I pursued it, I might have entered the Guinness book of records as the oldest graduate student alive that too without financial support. Anyway, this proposed problem required the understanding of two aspects of black holes: the geometrical structure of a black hole and the perturbations of its spacetime.

Geometry of black holes

Those were the early days when very little was known about black holes. Wheeler was going around giving his talk 'Gravitational Collapse: To What?' with missionary zeal. There was some vague notion of the metric component g_{00} of static spacetimes tending to zero on some surface. I distinctly remember the cold morning when, on the way to grab a sandwich at the little store run by the school of dairy research, Misner suggested that I look into this shady business. Fine, I said, thy will be done! There were false starts. I had this excruciating experience of translating to myself a lengthy paper in German by Ehlers and Sachs – or was it Ehlers,

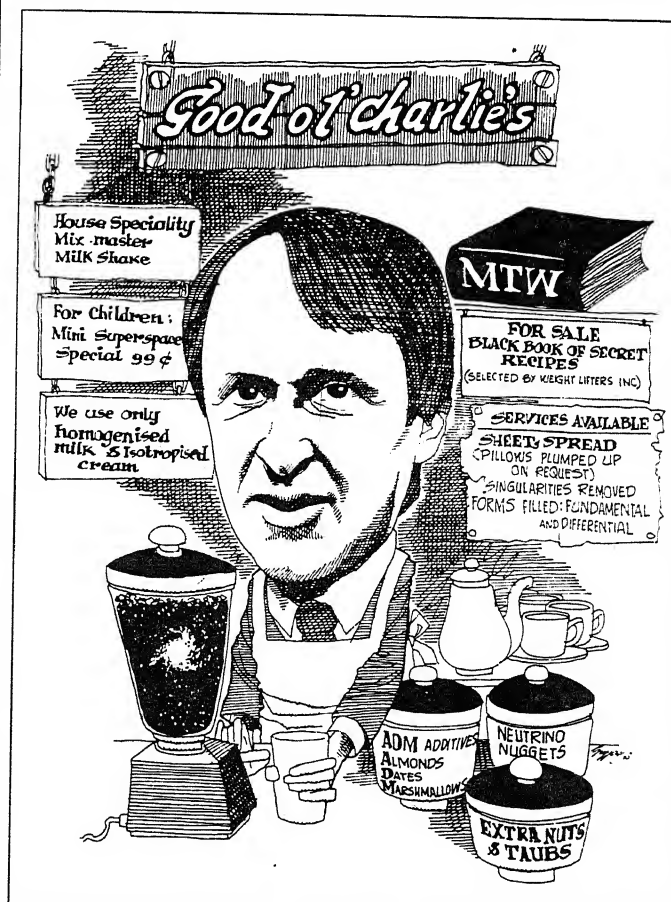
Kundt and Sachs, I forget – on ray optics and optical scalars in the hope that it would throw some light on the matter. It did not. Nevertheless, it was the article by Ehlers and Kundt¹ that gave the clue to the secret of the black hole structure. The covariant approach to the unravelling of the black hole geometry was via the spacetime symmetries or Killing vector fields. This way the fundamental properties of both Schwarzschild and Kerr black holes could be analysed, compared and contrasted. For instance, given a Killing vector field ξ one could derive the equation²,

$$n^a n_a = \frac{1}{2} [\xi^a \xi_a (\xi_{b;c} \xi^{b;c}) - \omega^r \omega_r], \quad (1)$$

where ω^a is the vorticity of the Killing congruence and n^a is the normal to surfaces of constant Killing norm, i.e.

$$\Sigma : \xi^a \xi_a = \text{constant}. \quad (2)$$

This shows that the surface on which ξ^a becomes null



C. W. Misner
The Master Mixer

Known for, among other things, his mix-master universe, cosmic isotropization by neutrinos, ADM formulation of general relativity, elucidation of the Taub-NUT spacetime, co-authorship of the black book *Gravitation*, and physics through spread sheets.

($\xi^a \xi_a = 0$), is itself a null surface, equivalently a one-way membrane or an event horizon ($n^a n_a = 0$), provided the vorticity also becomes null on the surface. The first condition implies that static observers cannot exist at and beyond this surface on which ξ^a is null. On the other hand, a null surface, satisfying the second condition, acts as a one-way membrane through which one can fall but cannot re-emerge from. This is the event horizon or the black hole. In the case of the Schwarzschild spacetime the surface on which the global timelike vector field ξ^a becomes null is a null surface since the vorticity of ξ^a is identically zero. In the case of Kerr spacetime, this is achieved for a suitable combination of the global timelike Killing vector ξ^a and the rotational Killing vector η^a . Consequently, the Schwarzschild black hole is both a one-way membrane and a static limit whereas in the Kerr spacetime, these two surfaces are distinct, thereby possessing the ergosphere between them. And this is where interesting phenomena like the Penrose process and the consequent energy extraction can occur.

Although the global timelike Killing vector field ξ^a of the Kerr spacetime possesses non-zero vorticity or rotation, Kerr spacetime admits an irrotational vector field

$$\chi^a = \xi^a - \frac{(\xi^b \eta_b)}{(\eta^c \eta_c)} \eta^a, \quad (3)$$

which is timelike down to the black hole. This vector field defines the Locally Non-Rotating Frames (LNRF)³, or the Zero Angular Momentum Observers (ZAMO)⁴. But this vector field exhibits much more interesting properties. These were investigated by Richard Greene, Englebert Schücking and myself⁵ around 1970. I had by then joined Schücking at New York University after a stint at the Institute for Space Studies of NASA in New York and a short period of unemployment. Perhaps it was too much to expect that black holes would be a source of income, since they were not sources of anything in the first place. To continue, we were able to generalize the irrotational vector field to arbitrary stationary, axisymmetric spacetimes with orthogonal transitivity. It was shown to be globally hypersurface orthogonal normal to $t = \text{constant}$ surfaces. These are maximal surfaces. The vector field could become null on an event horizon. Some features of this study, such as the physical interpretation of the mathematical conditions necessary for these properties, are still open problems. Incidentally, Iyer and I⁶ have recently renamed LNRF or ZAMO as GHOST – Globally Hypersurface Orthogonal Stationary Trajectories!

The geometry of Killing trajectories, i.e. the integral curves of Killing vector fields, that play such a basic role in elucidating the black hole structure, sneaked into our investigations in an indirect manner. Eli Honig was

studying the motion of charged particles in homogeneous electromagnetic fields using the Frenet–Serret (FS) formalism⁷. This formalism offers a geometric description of an arbitrary curve characterizing it by certain scalars and an orthonormal frame of reference at each point. In three dimensions these scalars are κ , the curvature and τ_1 , the torsion. In four-dimensional general relativity, we have an additional torsion τ_2 . Furthermore, the derivatives are with respect to proper time and, as a consequence, κ turns out to be the magnitude of four-acceleration. Similarly, the precession rate of a gyroscope carried along the curve has components τ_1 and τ_2 with respect to two members of the Frenet–Serret tetrad at each point of the curve. Now the worldliness of charges moving in a constant electromagnetic field F_{ab} bears striking resemblance to Killing trajectories. In both cases, each member of the FS tetrad satisfies the Lorentz equation. For Killing trajectories

$$F_{ab} = e^\psi (\xi_a \xi_b), \quad (4)$$

where the normalization factor $e^\psi = (\xi^a \xi_a)^{-1/2}$. In both cases, one can show κ , τ_1 and τ_2 are constants along the worldline and

$$\kappa^2 - \tau_1^2 - \tau_2^2 = \frac{1}{2} F_{ab} F^{ab}. \quad (5)$$

In the case of Killing trajectories, τ_1 and τ_2 turn out to be the components of vorticity. Further, acceleration is given by n_a , the gradient of equipotentials $\xi^a \xi_a = \text{constant}$, so that κ^2 is proportional to $n^a n_a$. With these substitutions, equation (5) reduces to equation (1). So, we have indirectly rederived the original black hole equation.

We shall return later to gyroscopic precession which we have mentioned here in passing.

Stability of the Schwarzschild black hole

The ultimate problem for my Ph D thesis, as mentioned earlier, was supposed to be the coalescence of two black holes. In order, at least, to make a beginning on this problem, I had to study perturbations superposed on the Schwarzschild spacetime as the background. The canonical paper in this area was, of course, the one by Regge and Wheeler⁸. To me this was a completely unknown territory. I remembered vaguely, a remark in Wheeler's book *Geometrodynamics*⁹ to the effect that the stability of the Schwarzschild spacetime was a problem far from having been solved satisfactorily. In fact, it was this book, totally incomprehensible to me when I was a first year graduate student in Columbia University and hence highly intriguing, that had drawn me towards general relativity. Another student of Misner, Lester Edelstein

and I rederived the perturbation equations and published them¹⁰ since these equations, as they appeared in the paper by Regge and Wheeler as well as at other places, contained errors. Lester was the first one to work out the radiation emitted by a particle falling into a black hole. He could not track down a factor of two that was missing when he compared his formula with the one of Landau and Lifshitz in the weak field limit. Unfortunately, as a result, he never completed his thesis and eventually switched from general relativity to actuaries. It was around this time that S. Chandrasekhar visited Maryland. It was a big event. Wheeler and his group, that included Uri Gerlach, Bob Geroch and Kip Thorne, drove down from Princeton. Chandra was getting interested in general relativity and in particular, black hole perturbations. We gave him our newly derived, yet unpublished equations. I had met Chandra a couple of years earlier at the Boulder Summer School in Colorado when I was aspiring to be a particle physicist in Columbia University. Other participating Indian students and I discussed with him our research as well as a bit of Indian science. In later years, I was to have the great privilege of having many discussions with Chandra on black hole physics, which became his chosen territory, and Indian science, in which he was keenly interested.

Stability analysis, as Ed Salpeter once put it, consists in finding out whether a system breaks apart if an ant sneezed in its vicinity. In the case of the black hole, the ant's sneeze is represented by metric perturbation which is a product of Fourier time mode $\exp(i\omega t)$, angular function which is a suitable tensor spherical harmonic and a radial function. Assuming the radial function to be well behaved, one had to show that imaginary frequencies that would make the perturbations grow exponentially in time were not admitted. Good behaviour had to be tested in reference to Kruskal coordinates that are singularity free at the black hole. Moreover, the radial functions corresponding to real frequencies had to be shown to form a complete set so that wave packets could be built that did not blow up in time. All this could be done for odd parity perturbations for which the radial function was governed by a Schrödinger-type equation with an equivalent potential. Frank Zerilli¹¹ would later derive a similar equation in the case of even parity perturbations. But, at the time, the even parity equation was a mess with frequency appearing all over the place. Stability analysis did not seem to go through. I was stuck, hopelessly stuck. Misner, who was going away to Cambridge for a year, suggested that I find a few simple, solvable problems and string them together into a thesis. My heart jumped into my mouth and my other organs rearranged themselves accordingly. I decided to devote another two weeks to the problem—body, mind and soul—and then quit if I did not make any progress. Those were the

days when Joe Weber was setting up his gravitational wave detector. Weber and his group were observing a rather peculiar phenomenon. Regularly around midnight the detector would record a sharp, beautiful peak. And then again another peak after an interval of a few minutes. Joe Sinsky, a graduate student, stayed on in the laboratory one night to investigate this puzzling phenomenon. Around midnight the door opened, a security guard came in and banged the door shut—the first peak. After making sure everything was secure in the laboratory he went out banging the door shut again—the second peak. Probably it was the same security guard who used to visit me around one in the morning. My working hours used to be from nine in the night to two in the morning. He would remove his shoes and his belt heavy with holstered gun, put up his feet on the desk and rest for a while. He would tell me what was going on in the world, including the parking lot, appreciate my working hard all alone through the night, sympathize with my non-existent wife waiting for me and then move on. On the eighth day from my decision to give stability a last try, my friend found me in a state of absolute euphoria. I had solved the problem. It had taken quite a bit of complicated analysis of the messy equation. Misner did not believe at first that the stability problem had been solved. But, after being convinced, he pronounced that my thesis was in the bag¹² and went away to Cambridge. And I, on my part, goofed off for one whole year.

Apart from establishing the stability of the Schwarzschild black holes¹³, the perturbation analysis had shown that the spacetime did not admit static perturbations that were regular at both the black hole and infinity. This was an indication that distorted static black holes could not exist in isolation. Nevertheless, it was startling to learn that Werner Israel had discovered the uniqueness of the Schwarzschild black hole¹⁴. There would be no potato-shaped black holes for instance. Nature had been robbed of her infinite variety. On the other hand, this clearly exhibited nature's simplicity. A static black hole could have only the shape of a sphere—the most perfect figure. After all, the philosopher Xenophanes, as early as in the sixth century BC, had declared that even God, being perfect, had to be spherical in shape!

Quasinormal modes

Halfway through the defense of my Ph D thesis, the examiner from the mathematics department asked the question, probably in a rhetorical vein, why one should bother to prove the stability of an object that was impossible to observe and was of doubtful existence in the first place. My thesis advisor did not like the question in the least especially coming from a mathe-

matician. The rest of the examination ended up as a verbal battle between the two which I watched with great satisfaction. But, the question remained: how do you observe a solitary black hole? To me the answer seemed obvious. It had to be through scattering of radiation, provided the black hole left its fingerprint on the scattered wave. I remembered from my first-year graduate course in quantum mechanics, how the reflection coefficient displayed maxima and minima in a wave scattered from a square barrier. In the case of the black hole also, the scattering was from a barrier, although of a different shape. So I thought, I might discover maxima in reflection coefficient characteristic of the black hole. In order to carry out this calculation you needed a computer, since the radial equation had to be numerically integrated. The days of the PCs were far in the future.

I was working at the Institute for Space Studies in New York where we did enjoy some luxuries. One of them was chilled beer that was sold at a quarter a can during seminars. So much so, the listeners soaked up more alcohol than astrophysics. The other luxury was computer time which was quite dear and scarce at other places. In addition, we had the help of a numerical analyst and a computer programmer. The reflection coefficient did show maxima albeit extremely faint. I became highly excited. But, when the range of integration was increased, the maxima shifted to some other frequency region. After quite a bit of computer experimentation, I decided that these were spurious maxima produced by the abrupt cut off of the effective potential. My conjecture was that a completely smooth potential would not give rise to maxima in the scattering cross section. I consulted Regge and Wheeler when I gave a talk at Princeton in 1969 with the alliterative title 'Schwarzschild Surface as a Stable Scattering Centre'. It was just before my seminar that I heard for the first time the term 'black hole' newly coined by Wheeler, which he illustrated with a picture of automobile junkyard he drew on the blackboard. Regge and Wheeler both agreed that there was no theorem connecting the smoothness of the potential to the non-existence of maxima in the scattering cross section. I still do not know the answer.

Although the scattering of monochromatic waves did not show obvious characteristics of the black hole, I felt that scattering of wave packets might reveal the imprint of the black hole. So, I started pelting the black hole with Gaussian wave packets. If the wave packet was spatially wide, the scattered one was affected very little. It was like a big wave washing over a small pebble. But when the Gaussian became sharper, maxima and minima started emerging, finally levelling off to a set pattern when the width of the Gaussian became comparable to or less than the size of the black hole. The final outcome was a very characteristic decaying

mode, to be christened later as the quasinormal mode. The whole experiment was extraordinarily exciting.

By the time the above work was published in *Nature*¹⁵, I had moved to New York University. Chandra made a visit and gave a talk on ellipsoidal figures of rotating fluids. He was very much interested in my work on scattering and in the phenomenon of decaying modes. Later on he was to compute the quasinormal mode frequencies with Detweiler¹⁶. Many calculations in this direction would follow finally culminating in the accurate determination of the frequencies by Nils Andersson¹⁷.

Quasinormal modes are generated in astrophysical scenarios such as gravitational collapse and coalescence of black holes. Ed Seidel has shown how well the fundamental mode matches the outgoing wave during the coalescence of binary black holes¹⁸. Recently Aguirregabiria and I have studied the sensitivity of the quasinormal modes to the scattering potential¹⁹. The motivation is to understand how any perturbing influence, such as another gravitating source, that might alter the effective potential would thereby affect the quasinormal modes. Interestingly, we find that the fundamental mode is, in general, insensitive to small changes in the potential, whereas the higher modes could alter drastically. The fundamental mode would therefore carry the imprint of the black hole, while higher modes might indicate the nature of the perturbing source.

Quasinormal modes are perhaps the rebuttal to the criticism of my thesis examiner regarding the nonobservability of black holes.

Ultracompact objects

One of the indirect offshoots of black hole research was the study of ultracompact objects or UCOs. While investigating the scattering of gravitational waves from the Schwarzschild black hole, I had noticed a peculiar phenomenon which I did not publish. Although at the time, the radial equation for even parity perturbations was quite complicated and was not in the Schrödinger form, it yielded exactly the same reflection coefficient as the odd parity perturbation for a given angular parameter l . One day I got a very excited telephone call from Chandra enquiring whether I knew this fact. I answered, yes, I did. Did I know why this happened? No, I did not. He had found the reason, he said triumphantly. He asked me for the numbers I had computed which I sent him. He went on to publish his interesting conditions under which two potentials lead to identical scattering cross sections mentioning my foreknowledge of the fact but not the reason.

It is the same story with neutrinos as well. The two equivalent potentials corresponding to the two helicities are quite different from each other²⁰, but lead to identical

reflection coefficients. One of them has the peculiar feature in that it has a potential well in the region $r < 3m$ attached to the usual potential barrier. This was terribly intriguing. Could there be a bound state in the potential well giving rise to some sort of neutrino trapping by the black hole? One can estimate the maximum number of bound states by integrating the potential over its spatial range. The well depth increases with the angular momentum quantum number and in the limit of its tending to infinity you get the answer one for the maximum number of possible bound states. In other words, there are no bound states.

I did not publish any of the above results. But out of it all another interesting question arose. Suppose you replaced the black hole by a spherical star of radius $r < 3m$. Then the potential well would not only exist, but would also be deepened by the enhanced gravitation of the matter. Could there then be bound states trapping neutrinos within the star? Ajit Kembhavi and I worked on this problem, found and computed the complex frequencies corresponding to the bound states of the neutrinos²¹. In a way, these neutrino bound states with complex frequencies were forerunners of the quasinormal modes of ultracompact stars worked out by Chandrasekhar and Ferrari²² as has been pointed out by Andersson²³. It is a very happy feeling that some of the problems I had worked on interested Chandra also.

Ultracompact objects with radius $r < 3m$ are in fact quite interesting entities. In principle, trapping of massless particles in their potential well is possible. Or the object can oscillate in its quasinormal modes. Van Paradis²⁴ has pointed out the peculiar behaviour of redshift for $r < 3m$. Recently, Abramowicz and Prasanna²⁵ have discussed the reversal of centrifugal force at $r = 3m$ for which you need a black hole or a UCO.

But, do such highly compact objects or stars with radius $r < 3m$ exist in nature? This question was considered by Dhurandhar, Iyer and myself^{26,27} and we also coined the name 'Ultra-Compact Objects' or 'UCOs'. By studying very carefully the general relativistic stellar models with different equations of state we established that, as a matter of fact, stable ultracompact objects can exist in nature.

Gyroscopic precession and inertial forces

We discussed earlier how the black hole structure can change dramatically when going from static to stationary spacetimes on account of the rotation inherent to the latter. The study of Killing trajectories in these spacetimes led to a covariant description of gyroscopic precession via the Frenet-Serret formalism. Precession is an important phenomenon. For instance, the Earth precesses. Ancient astronomers knew this. Astrologers did not,

thereby making predictions that were doubly wrong. Or, can two wrongs add up to one right? In atomic physics, Thomas precession, a manifestation of special theory of relativity, played a crucial role. Spacetime curvature gives rise to Fokker-De Sitter precession in the Schwarzschild spacetime. The spin of Kerr black hole contributes additional precessional effects. All these can be studied elegantly using the Frenet-Serret description⁶.

Another related area concerns the general relativistic analogues of inertial forces as developed by Abramowicz and coworkers²⁸. A particle at rest in a static spacetime experiences only the gravitational force, but is acted upon by the centrifugal force as well if it is moving uniformly in a circular orbit. In a stationary spacetime, there is an additional force, the Coriolis-Lense-Thirring force, which arises as a consequence of the metric components mixing space and time. In static spacetimes, such as the Schwarzschild spacetime, centrifugal force reverses at the circular photon orbit²⁵. So does gyroscopic precession. The situation is far more complicated in stationary spacetimes^{29,30}. My young colleague Rajesh Nayak and I have studied these effects and established covariant connections between gyroscopic precession on the one hand and inertial forces on the other³¹⁻³³. These considerations should be of interest in black hole physics from a conceptual point of view as well as for astrophysical applications.

The trail goes on . . .

I have tried to offer a glimpse, just a fleeting one at that, of my personal journey along the black hole trail. It has been a long journey spanning some three decades. There have been all sorts of ups and downs along the way. For instance, I have had my share of tussle with journals and referees. My very first paper¹⁰, the one with Edelstein, was unceremoniously rejected as nothing more than a bunch of formulae. Misner had to write a strong letter pointing out that the same journal that had previously published the wrong equations was now rejecting the correct ones. The paper on the structure of black holes² was also rejected as it was considered to be just mathematics and had to be published in the *Journal of Mathematical Physics*. The stability paper¹³ too had to cross some hurdles before seeing the light of the day. As with any important field, black hole physics has had its sociological factors sometimes leading to, among other things, inadequate recognition of significant contributions. All this becomes trivial in comparison to the exhilarating experience of exploration. It is a rare good fortune to have been trekking along the track right from the beginning. To have watched the seed germinate, the sapling sprout and the tree grow. It is also a good fortune to have had the company of

congenial co-travellers on the journey – marvellous friends to work with and keen minds to lead the way. If sometimes you stray away from the road, you keep coming back. Even now my colleagues and I are working on different aspects of black hole physics, such as quasinormal modes, rotational effects and black holes in cosmological backgrounds.

What is the most important lesson I have learnt having traversed the trail for so long? Let me answer that question by quoting the Spanish poet Antonio Machado, who wrote:

*Caminante, no hay camino
Se hace camino al andar.*

Traveller, there is no path,
Paths are made by walking.

It is gratifying, to feel that you have made a path however short, however narrow that has helped build a trail that was planned and paved by so many. It has been a joy to follow that trail. And I hope the trail will never end.

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JGOFS (India) – Introduction

The Joint Global Ocean Flux Study (JGOFS) is a core project of the International Geosphere–Biosphere Programme (IGBP), the main international scientific forum addressing issues pertaining to global change. The scientific goals of JGOFS were put together about a decade ago under the auspices of the Scientific Committee on Oceanic Research (SCOR). The main goals of JGOFS are:

- To determine and understand on a global scale the processes controlling the time-varying fluxes of carbon and associated biogenic elements in the ocean, and to evaluate the related exchanges with the atmosphere, the sea floor, and continental boundaries.
- To develop a capability to predict on a global scale the response of oceanic biogeochemical processes to anthropogenic perturbations, in particular those related to climatic change.

To accomplish these goals a detailed implementation plan with specific scientific objectives have been formulated. One of the key components of this plan is *process studies* in selected *biogeochemical provinces* in the oceans which can significantly influence the global carbon budget. Among the biogeochemical provinces identified by the international JGOFS community, one of them is the Arabian Sea known for its high and seasonally oscillating biological productivity resulting from monsoon-driven circulation. In addition, the northern regions experience enhanced biological productivity in winter which has been attributed to convective overturning associated with winter cooling. One of the goals of the JGOFS programme is to assess the variations in primary and new production and the flux of carbon through the water column during different seasons, especially that arising from the monsoonal circulation. The effect of these physical and biological processes (upwelling, biological productivity) on the CO₂ air–sea exchange fluxes, carbon cycling in the water column and its burial in the sediments is not well understood and is a topic of considerable interest and debate among oceanographers. The international JGOFS community during the past 3–4 years have been conducting major campaigns in this oceanic region, particularly in the monsoon-induced upwelling areas of the western and central Arabian Sea to address the above issues.

The JGOFS (India) programme was seeded during the international symposium on the 'Oceanography of the

Indian Ocean' held at the National Institute of Oceanography, Goa, January 1991. The Department of Ocean Development constituted a Steering Committee to generate, monitor and implement JGOFS programme in India. An interdisciplinary, multi-institutional project was formulated for JGOFS-related studies in India with focus on central and eastern Arabian Sea. Broadly the goals of the JGOFS (India) programme are (i) to assess the role of the Arabian Sea in the overall CO₂ air–sea exchange balance by determining the magnitude and direction of CO₂ air–sea exchange fluxes during different seasons; (ii) to determine the primary and new production rates, their spatial and temporal variations, the export flux of carbon from the euphotic zone and its relation to primary productivity, (iii) study of the records of deposition of carbon and other elements in the sediments of the Arabian Sea, particularly in its margin and their relation to water column processes; (iv) to investigate the role of margin sediments in influencing the chemistry of the Arabian Sea interior. Scientists from the National Institute of Oceanography (Goa), Physical Research Laboratory (Ahmedabad), National Chemical Laboratory (Pune) and Centre for Mathematical Modelling and Computer Simulation (Bangalore) are participating in JGOFS (India) programme.

The JGOFS (India) programme is currently in its mid-way. During the last two years it has completed three major sampling expeditions to the eastern and central Arabian Sea. The first cruise (SK-91) was undertaken during the pre-monsoon season, April–May, 1994. This was followed by a winter cruise (SK-99, February–March, 1995) and a monsoon cruise (SK-104, July–August, 1995). All these expeditions by and large followed the same track, however, sampling in the northern latitudes could not be done during the monsoon cruise because of logistical problems. In addition, a fourth expedition is currently underway (August, 1996) and a fifth one is planned for January, 1997.

This special section of *Current Science* contains the first collection of articles of the JGOFS (India) programme, most of the papers deal with the results from the intermonsoon (SK-91) and winter cruises (SK-99). The articles by S. Prasanna Kumar and T. G. Prasad (page 834) and by P. M. Muraleedharan and S. Prasanna Kumar (page 842) provide a comprehensive overview of the physical oceanography of the central and eastern Arabian Sea, including the near-coastal regions. Their results suggest that during winter, the northern Arabian

Sea surface waters experience densification due to excess evaporation over precipitation and turbulent heat loss in excess of radiative heat gain. This causes sinking and convection which pumps nutrients into surface waters from deeper layers, enhancing biological productivity. These studies demonstrate for the first time through direct observations the effect of winter cooling on the mixed layer dynamics of the northern Arabian Sea and associated enhancement in biological productivity.

The spatial and temporal variations in primary productivity and chlorophyll distribution are addressed in the article by P. M. A. Bhattathiri *et al.* (page 857). They observe that during winter the primary production and Chl *a* abundances in the northern latitudes were about a factor of two higher than those during the preceding intermonsoon season, consistent with that expected from changes in circulation. The highest productivity measured in this region ($\sim 800 \text{ mgC m}^{-2} \text{ d}^{-1}$) is about a factor of 2–3 lower than that reported for the north western Arabian Sea during the SW monsoon, but is similar to that observed in the north Atlantic during the JGOFS North Atlantic Bloom Experiment.

Fundamental to all biological processes is the nature and availability of solar radiation in the ocean. This important aspect has been studied by T. Suresh *et al.* who measured (February–March, 1995) the photosynthetically available radiation in the eastern and central Arabian Sea. The peak values observed were in the range of 365 to 435 W m^{-2} . In addition, aerosol optical depth measurements yielded values from 0.07 to 0.19. These measurements have great relevance to remote sensing, validation of algorithms and models, and the peculiarity of the Arabian Sea where oscillation of high rates of primary productivity under more or less constant levels of solar radiation take place.

The articles by S. Sawant and M. Madhupratap (page 869), M. Madhupratap *et al.* (page 863), N. Ramaiah *et al.* (page 878) and M. Gauns *et al.* (page 874) discuss various issues pertaining to the abundance and distribution of phytoplankton and zooplankton, bacteria and micro-zooplankton respectively. The abundance of bacteria decreased drastically from intermonsoon to the winter season. The high abundance during the low productive intermonsoon period is probably sustained by the dissolved organic pool that may build up as the bloom tapers off. The standing stock of bacterial carbon during intermonsoon was significantly higher than that of phytoplankton, thus contributing to much of the POC in the region. The mezo-zooplankton abundance studies attest to the Arabian Sea paradox of its general invariance with productivity. This paradox, most likely results from the switch over of the feeding pattern of the mezo-zooplankton, from phytoplankton during productive sea-

sons to microbial loop during oligotrophic periods. This is also attested by the observation that microzooplankton carbon was higher than meso-zooplankton standing stock.

The influence of water circulation and biological productivity on the nutrient and oxygen distribution in the water column are presented by S. N. De Sousa *et al.* (page 847). During winter, intense reducing conditions occur in the intermediate waters resulting from sluggish water movement coupled with higher biological productivity. The studies revealed the occurrence of nitrate reduction in all seasons in sub-oxic waters, which is maximum (10 μm) in winter.

The measurements of the air–sea exchange fluxes of the greenhouse gases, CO_2 , CH_4 and N_2O are presented in the articles by V. V. S. S. Sarma *et al.* (page 852) and Shyam Lal *et al.* (page 894). The pCO_2 values in surface waters were generally higher or equal to those in the atmosphere, with values of about 420 μatm during winter and between 360 and 420 μatm during intermonsoon and monsoon seasons. These results suggest that the central and eastern Arabian Sea serve as a source of CO_2 to the atmosphere, typical flux being \sim a few $\text{mmoles m}^{-2} \text{ d}^{-1}$. Analogous to CO_2 , this region of the Arabian Sea also supplies CH_4 and N_2O to the atmosphere, with higher fluxes during winter. The next step in these studies should be to have a better understanding of the various water column processes contributing to the production and consumption of these gases which would enable development of models to quantify their air–sea fluxes.

The application of ^{234}Th : ^{238}U disequilibria in the surface waters to derive information on particle and carbon export fluxes and time scales solute–particle interactions are discussed by M. M. Sarin *et al.* (page 888). They observe that the residence time of chemically reactive elements like Th in surface waters of the Arabian Sea is about a month. More importantly, these authors observe that the export flux of carbon at 100 m, estimated from ($^{234}\text{Th}/\text{C}$) ratios measured in sediment trap materials and ^{234}Th deficiency in the water column is significantly higher than primary productivity. These results raise concern about the source of carbon to the traps and the underlying assumptions in using ^{234}Th as a survey tool for estimating carbon export.

Another important area of study of the JGOFS (India) programme is the sedimentary record, which provide data on the temporal variations in the burial fluxes of several biogenically important elements, and their relation to water circulation and productivity history. A large number of sediment cores from the continental margin of the Arabian Sea have been collected and are being analysed for a number of diagnostic tracers to decipher the environmental history of sediment deposition and

their role in regulating the distribution/removal of trace elements in the water column. The article by D. N. Yadav (page 900) addresses to one of the issues, viz. cycling of carbon in the sediments mediated through manganese.

Data management and modelling are two other areas of study which were initiated as a part of JGOFS (India) programme last year. The data and results of JGOFS (India) programme are stored and managed at the Indian National Oceanographic Data Centre (INODC) at NIO, Goa. Scientists from the Centre for Mathematical Modelling and Computer Simulation, Bangalore are in the process of developing a coupled basin scale model of the Arabian Sea circulation and biogeochemical cycles.

The results of these process studies hopefully will be incorporated into predictive models. However, long term continuous time series observations using moored data buoys with sensors which measure selected parameters would have to be deployed in the Arabian Sea to fully understand the biogeochemical processes. Ideally this should be supplemented by remote sensing techniques. Future work would thus have to be in this direction. In addition, work similar to the JGOFS could be initiated in the Bay of Bengal, which has its own unique and special oceanographic characteristics and which necessarily has to be investigated if one aspires to predict processes on a global scale.

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Winter cooling in the northern Arabian Sea

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The upper thermo-haline structure and the surface meteorological parameters of the central and eastern Arabian Sea during the inter-monsoon (April–May, 1994) and winter monsoon (February–March, 1995) periods, were analysed to understand physical forcing that leads to the observed high productivity during winter in the northern Arabian Sea. The weak northerly winds and increased solar insolation during the inter-monsoon period, led to the development of a highly stratified upper layer with warm sea surface temperature (SST) (29.5°C) and shallow mixed layer depth (MLD) (25 m). In contrast, during winter, the upper thermo-haline field showed a dramatic change, with cold SST (25°C) and deep MLD (100 m) in the north, though the winds were weak (5 ms⁻¹). The atmospheric forcing that leads to the observed changes in the upper layer of the ocean is a combination of enhanced evaporation under the influence of the dry continental air from the north brought by the prevailing northeasterly trades and reduction in the solar insolation. Subsequent cooling and convective mixing injects nutrients into the surface layers from the thermocline region, which in turn triggers the primary production.

It is well known that during summer monsoon (June–September) upwelling is a dominant mechanism which triggers plankton bloom and results in extremely high productivity in the Arabian Sea^{1,2}. In contrast, the spatial variation in the productivity during winter (November–February) is less studied. On the basis of analysis of published data, Banse³ inferred that the central Arabian Sea is more productive than subtropical gyre elsewhere, and the area north of 20°N to be richer than the central Arabian Sea. Our results from a recent Indian Joint Global Ocean Flux Studies (JGOFS) cruise, during February–March 1995, revealed the evidence of high productivity in the northern Arabian Sea, the details of which are presented elsewhere, in this issue⁴. In this article, we analyse the upper thermo-haline structure and the physical forcing that lead to the observed high productivity.

Data and methods

As a part of the Indian JGOFS programme, 36 CTD (Conductivity Temperature Depth) profiles were collected at one degree interval (up to 1000 m depth) onboard ORV *Sagar Kanya* during 4 February to 4 March 1995 (Figure 1a). In accordance with the

JGOFS protocol⁵, Sea-Bird Electronics CTD was used to obtain temperature and salinity profiles. CTD salinities were calibrated against water samples collected simultaneously by a rosette sampler and analysed with a Guildline 8400 Autosol. The conversion of conductivity to salinity was made using the modified UNESCO formula⁶. The data collected during the JGOFS cruise of 14 April to 14 May, 1994 (Figure 1b) were also utilized to highlight the changes in the water column. Apart from the CTD profiles, surface meteorological parameters were also collected during both the cruises. The surface meteorological observations on a 2° × 2° grid from the Comprehensive Ocean Atmosphere Data Set⁷ (COADS) were used to obtain monthly mean values of surface heat flux and evaporation. The surface fluxes (Wm⁻²) were calculated using standard bulk aerodynamic formulae⁸ for latent (Q_e) and sensible (Q_h) heat exchange:

$$Q_e = \rho L C_e (q_s - q_a) U,$$

$$Q_h = \rho C_p C_s (T_s - T_a) U,$$

where L is the latent heat of vaporization, C_p the specific heat of water, q the specific humidity, C_e and C_s the empirical exchange coefficients, T_s the sea surface temperature, T_a the air temperature, U the wind speed. The evaporation is computed by using the equation

$$E = \rho C_e (q_s - q_a) U.$$

The precipitation data were obtained from the Very High Resolution Radiometer (VHRR) onboard the INSAT (see ref. 9 for details). From these data, monthly mean precipitation on a 2° × 2° grid was generated. Further, monthly values of evaporation–precipitation (E–P) were computed along 64°E.

Results

To understand the physical process, we analysed the observed surface wind as well as the vertical structure of temperature, salinity and sigma- t along the 4 legs (Figure 1).

Winds

The surface winds during February–March (Figure 2a) were predominantly north/north-easterlies with occasional north-westerlies. Along the shelf, the observed

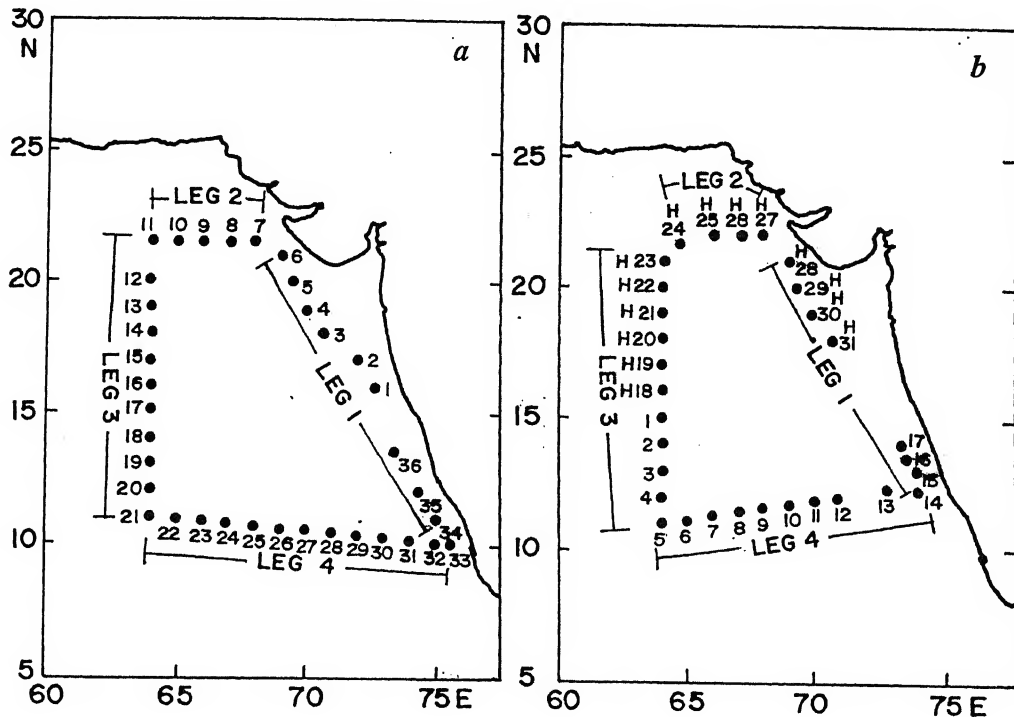


Figure 1 *a, b*. Location map of CTD stations occupied during (*a*) 4 February to 4 March (winter monsoon) 1995 and (*b*) 14 April to 14 May (inter-monsoon) 1994. H indicates profiles taken with hydrocast.

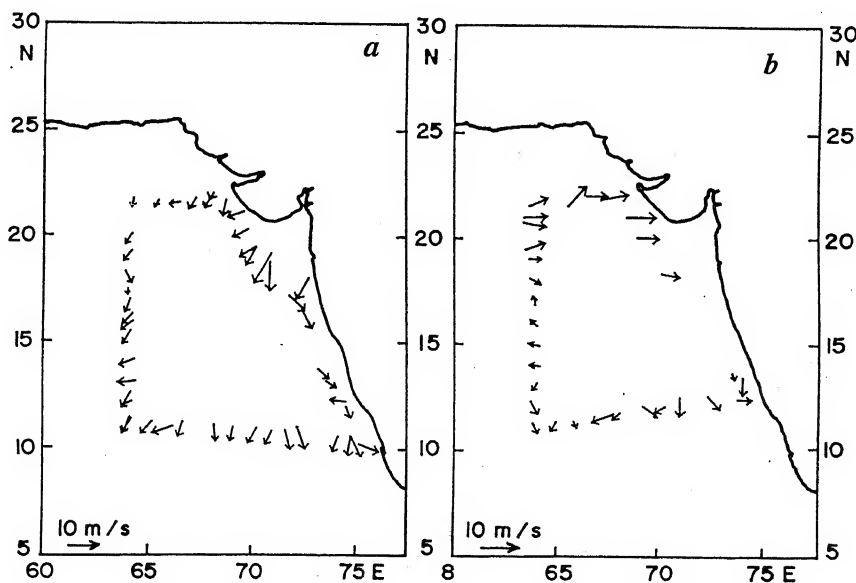


Figure 2 *a, b*. Wind vector during (*a*) winter monsoon, (*b*) inter-monsoon.

wind speed varied between 4 and 7 ms^{-1} , except at 18°N where it was about 12 ms^{-1} . In the open ocean, the wind speed showed a gradual increase from about 2 ms^{-1} to 5 ms^{-1} towards south. During April–May (Figure 2 *b*) the surface winds south of 17°N were predominantly

northerly and weak (<4 ms^{-1}), but became progressively stronger towards north. Along the northern shelf, the wind speeds were between 8 and 10 ms^{-1} , decreasing to 6 ms^{-1} towards south. In the open ocean, wind speeds were high north of 18°N reaching up to 8 ms^{-1} .

Thermo-haline characteristics

Winter monsoon (February–March)

The thermal structure during February–March, along the shelf (leg 1), (Figure 3, top panel) showed a weakly stratified surface layer extending to 100 m depth, and the sea surface temperature (SST) rose from about 24.2°C in the north (21.5°N) to about 29°C at 10°N, approximately 0.5°C increase per degree latitude. The mixed layer depth (MLD), in general, was deep varying between 120 m at 21°N and about 80 m at 10°N. The packed isotherms below 100 m, along the southern shelf, indicated a strong thermocline. Salinity structure (Figure 3, bottom panel) also showed a weakly stratified layer of high salinity (36.4 PSU), in the north, thinning towards south. The very low salinity waters (< 34.6 PSU) towards south indicate the influence of the Bay of Bengal waters, being carried along the shelf by northward flowing coastal current. The high salinity surface waters (36.4 PSU) in the north is the Arabian Sea High Salinity Water mass (ASHSW), the core of which deepens towards south (about 100 m depth) and was identified by the 36.0 PSU isohaline.

Along 64°E (leg 3), the thermal structure showed a weakly stratified surface layer extending to 100 m

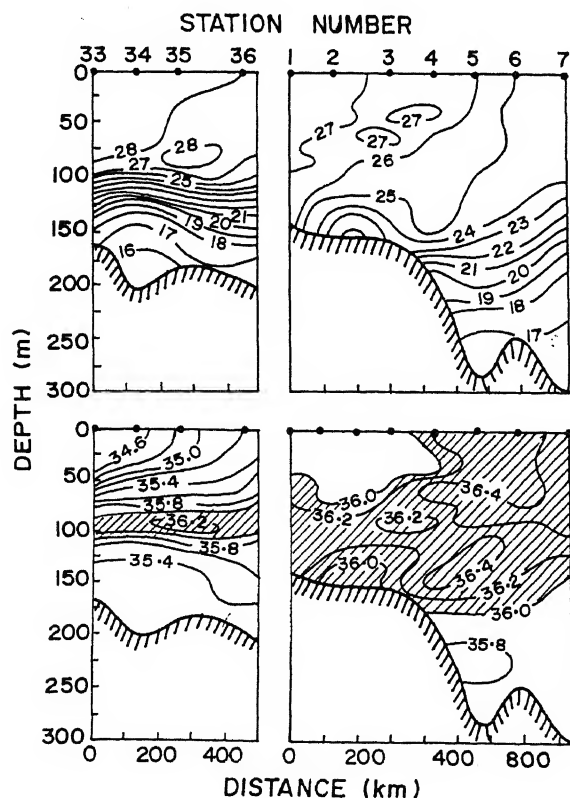


Figure 3. Vertical section of temperature (top) and salinity (bottom) along the shelf during winter monsoon. Salinity >36 PSU is hatched to demarcate the Arabian Sea High Salinity Water mass.

depth, as seen along the shelf, with colder waters (<25°C) north of 17°N, while south of it, the SST increased to 27°C (Figure 4, left panel). Thermal structure depicts deep mixed layer in the north (21.5°N) which shoals to about 80 m at 20°N and once again deepens to 125 m at 17°N. South of 17°N, mixed layer depth shoals steadily, reaching up to 65 m at 11°N. Isotherms below the upper thermocline showed considerable shoaling towards south giving rise to strong gradient in the lower thermocline as well. This thermal structure essentially reflects the zonal flow pattern in the open ocean circulation. Below 500 m, the waters are well stratified with no appreciable north–south variation. Salinity structure (Figure 4, middle panel) showed the presence of ASHSW near the surface (> 36.4 PSU) in the northern region, the core of which deepens towards south. The surface salinity decreased from 36.4 PSU in the north to less than 35.7 PSU at 11°N. This low salinity water is the indication of North Equatorial Current (NEC), which carries along with it the low salinity waters from the Bay of Bengal as well as the eastern Indian Ocean into the western Arabian Sea during this season. The second subsurface high salinity (36.0 to 36.2 PSU) seen between 200 and 400 m in the north and spreading southward is the Persian Gulf water mass, which was also seen spreading eastward along leg 2: As it moves southwards beyond 14°N, the water mass loses its identity due to mixing with the Red Sea water mass, normally encountered south of 13°N, between 400 and 800 m depth and sigma- t 27.0, which is manifested by the thick isohaline layer between 200 and 800 m depth near 11°N. The sigma- t structure (Figure 4, right panel) mainly reflects the combined effects of thermo-haline structure. In the northern region, sigma- t greater than 24.5 was encountered at the surface with thick isopycnal layer (100 m), which gradually decreases southward and becomes as low as 22.0

The MLD along the zonal section (nominally along 10°N, leg 4), increased gradually from about 50 m in the west to almost 100 m towards the east with SST in general, warmer than 28°C (Figure 5, left panel). The packed isotherms in the upper 300 m indicate the signature of the NEC active during the winter monsoon. The presence of NEC brings about drastic changes in the surface salinity along this section (Figure 5, right panel) with very low salinity waters (34.6 PSU) from the eastern side over-riding the high salinity waters of the west. The core of the ASHSW (36.0 PSU) consequently showed an eastward deepening. The thick isohaline layer between 200 and 1000 m (which thins towards east), once again is the admixture of Persian Gulf and Red Sea waters.

Inter-monsoon (April–May)

Along the western shelf (leg 1), thermal structure

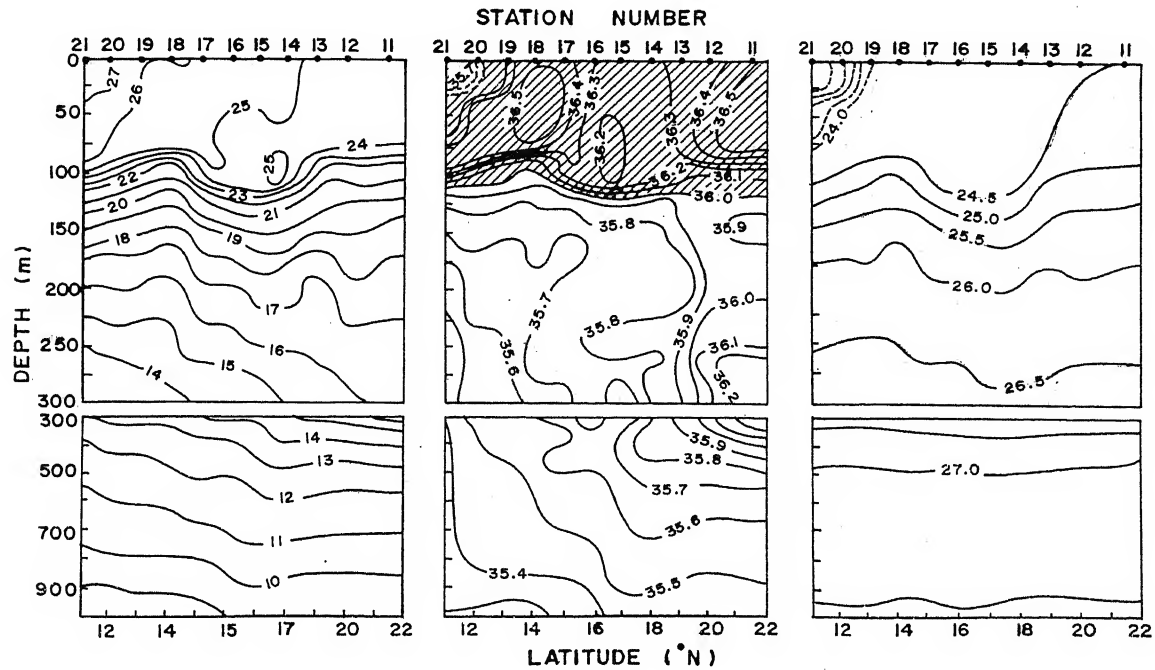


Figure 4. Vertical section of temperature (*left*) salinity (*middle*) and sigma-*t* (*right*) along 64°E during winter monsoon. Salinity >36 PSU is hatched to demarcate the Arabian Sea High Salinity Water mass.

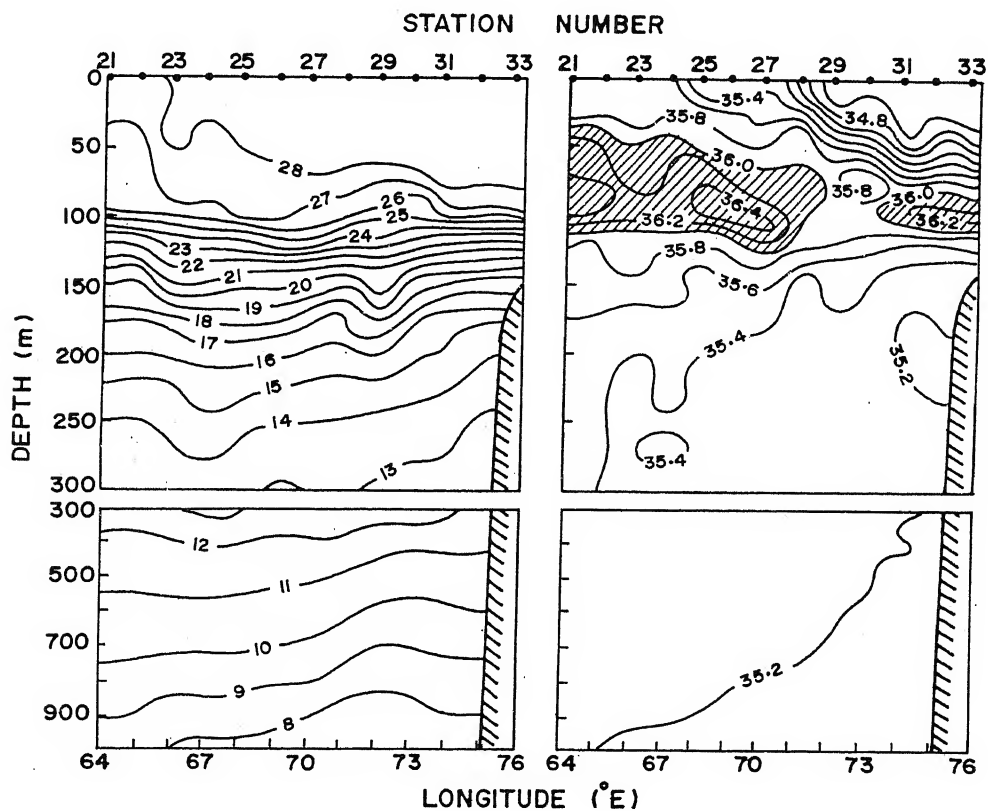


Figure 5. Vertical section of temperature (*left*) and salinity (*right*) nominally along 10°N during winter monsoon. Salinity >36 PSU is hatched to demarcate the Arabian Sea High Salinity Water mass.

showed thin mixed layer, varying marginally from 10 m in the north to 25 m in the south, with SST more than 30°C (Figure 6, top panel). More or less uniformly thick, high salinity waters (36.4 PSU) are encountered along the northern shelf, north of 16°N (Figure 6, bottom panel). However, along the southern shelf, low salinity waters are seen in the upper 20 m. The core of the ASHSW, which is seen almost at the surface in the north, deepens to about 80 m towards south.

The thermal structure along 64°E (leg 3) showed a well-stratified surface layer. The upper thermocline showed an overall trend of deepening from 21°N to 11°N, except between 13.5°N and 15.5°N where isotherms greater than 20°C shoals, appear to be a signature of a meso-scale (cold core) eddy (Figure 7, left panel). The MLD remained thin showing a general deepening, though small, toward south as in the case along the western shelf (leg 1). Salinity structure resembled that during February–March, except for the reduced spatial extent of the low salinity water in the southern region (Figure 7, middle panel). The thick isohaline layer seen in the north, during February–March, is no longer present. Instead, the high salinity (36.6 PSU) protrudes into the water column, indicating the spreading and deepening of the ASHSW towards south. Unlike during February–March, the sigma- t structure showed the presence of low density waters uniformly throughout from 21°N to 11°N (Figure 7, right panel) and strong stratification. The 24.5 isopycnal seen at the surface in the north during winter monsoon is not encountered at 60 m.

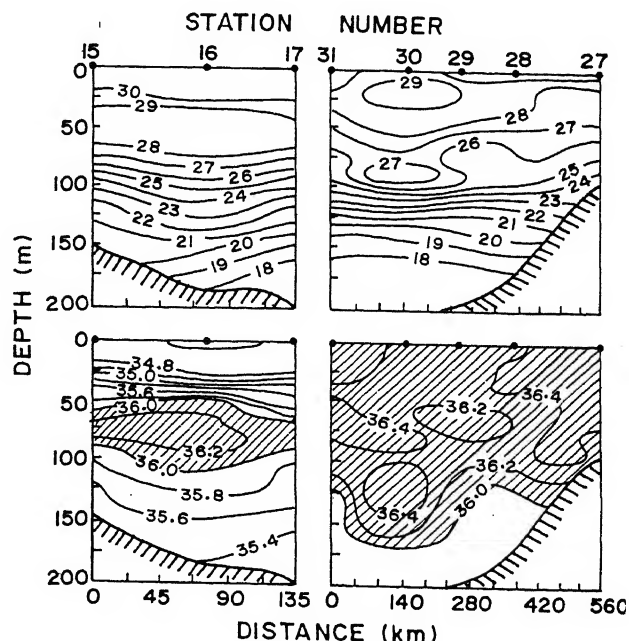


Figure 6. Vertical section of temperature (top) and salinity (bottom) along the shelf during inter-monsoon. Salinity >36 PSU is hatched to demarcate the Arabian Sea High Salinity Water mass.

Along leg 4 (nominally along 10°N), thermal structure showed extremely shallow MLD in comparison with February–March with the mixed layer temperature

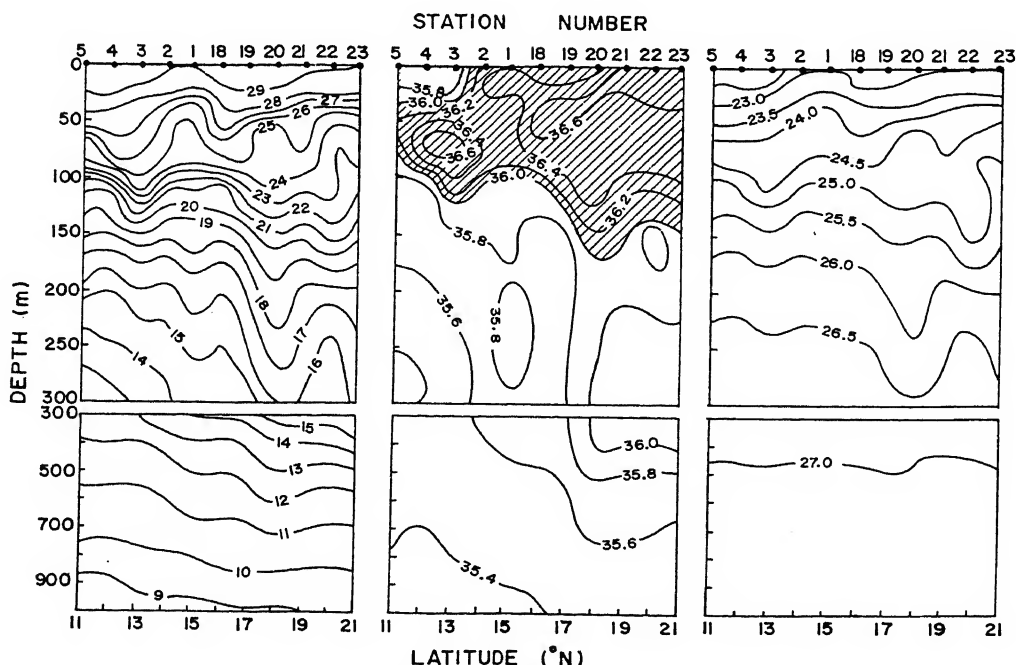


Figure 7. Vertical section of temperature (left) salinity (middle) and sigma- t (right) along 64°E during inter-monsoon. Salinity >36 PSU is hatched to demarcate the Arabian Sea High Salinity Water mass.

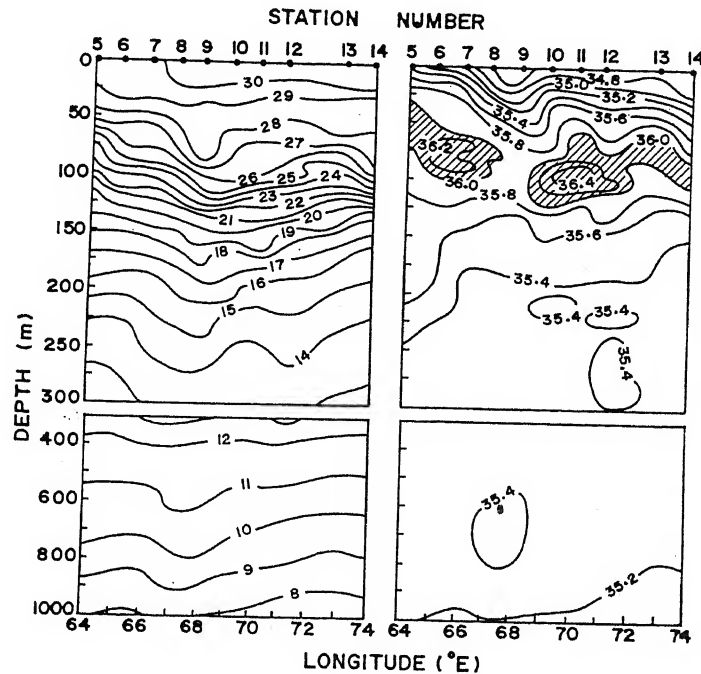


Figure 8. Vertical section of temperature (left) and salinity (right) nominally along 10°E during inter-monsoon. Salinity >36 PSU is hatched to demarcate the Arabian Sea High Salinity Water mass.

at least 2°C warmer (Figure 8, left panel). Though, in general the salinity structure was similar to that during February–March, the haline gradient seen at the eastern region is diffused (Figure 8, right panel). The thickness of the ASHSW also showed considerable change.

Surface meteorological parameters

To highlight the changes of the top layer of the ocean during winter monsoon, we over-plotted the air temperature, SST and MLD of inter-monsoon period along 64°E (leg 3).

The air temperature, during winter monsoon, increased from 23°C in north to a little more than 26°C in the south, while it was, in general, more than 28°C during inter-monsoon, and at times reached up to 30°C (Figure 9 a). The SST showed about 3°C rise from north (25°C) to south, in winter as compared to 1°C rise in inter-monsoon (Figure 9 b). The MLD during winter was deep (100 m) in the north (Figure 9 c), which shoals up to 80 m at 20°N and once again deepens to 120 m at 17°N. South of this MLD shoals steadily reaching up to 65 m. In comparison to winter monsoon, MLD was very shallow, varying between 10 m in the north and 35 m towards south, during inter-monsoon.

To understand the atmospheric forcing that lead to the observed changes in the upper thermo-haline fields, we analysed the heat and fresh water (evaporation–precipitation) fluxes along 64°E.

The time-latitude distribution of the latent heat flux along 64°E showed two high values, one during November–January, specially north of 10°N (130 Wm⁻²), and the other during May–August (220 Wm⁻²) in the central Arabian Sea (Figure 10 a). The sensible heat flux showed heat gain in the central Arabian Sea from June

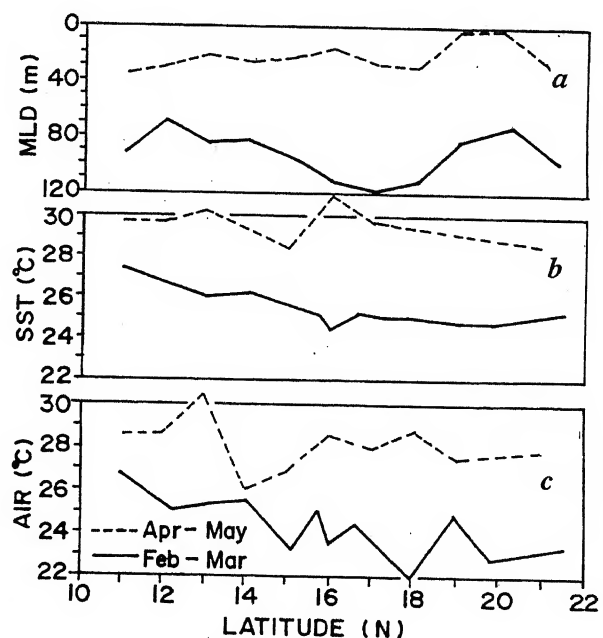


Figure 9 a-c. Distribution of (a) air temperature, (b) SST and (c) MLD. The solid line indicates winter monsoon and broken line inter-monsoon.

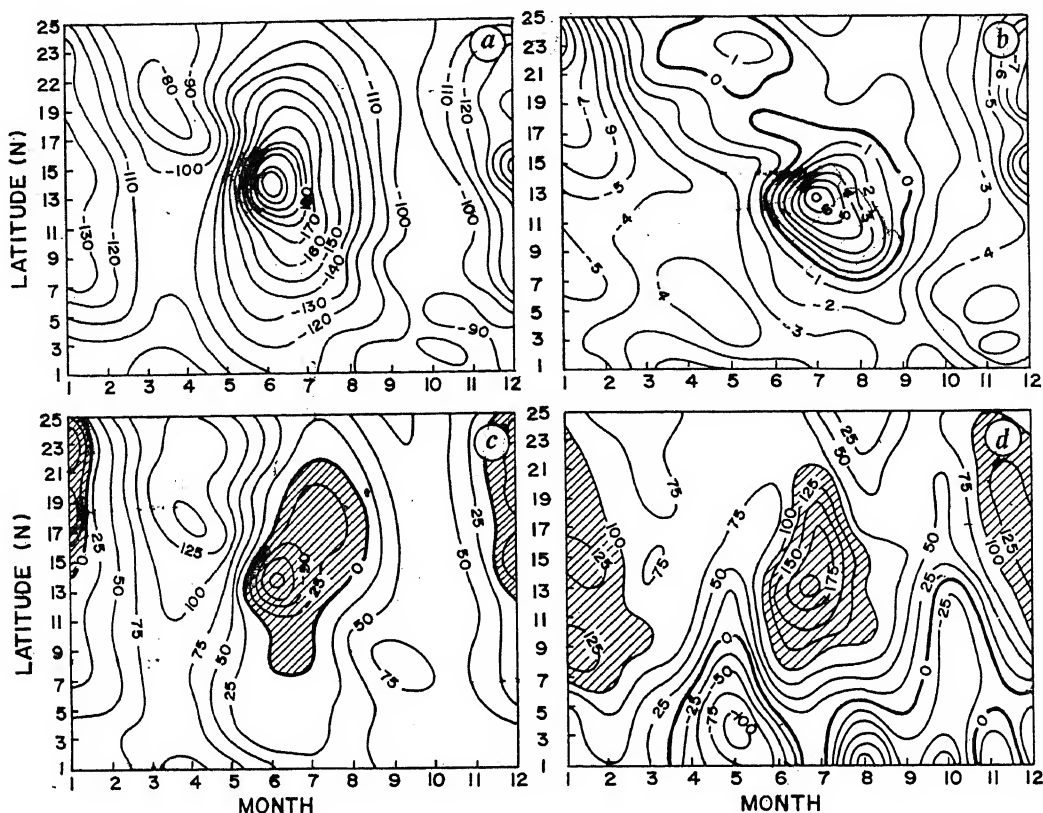


Figure 10 *a-d*. Time-latitude section of (*a*) Latent heat flux (Q_e , Wm^{-2}), (*b*) Sensible heat flux (Q_s , Wm^{-2}), (*c*) Net heat flux (Wm^{-2}), and (*d*) evaporation-precipitation ($E-P$, mm/month) along 64°E . The hatched region in (*c*) represents a net heat loss while that at (*d*) shows where evaporation exceeds precipitation by more than 100 mm/month .

to September, while the northern Arabian Sea experienced significant heat loss during winter monsoon (about 7 Wm^{-2} , Figure 10 *b*). The net heat flux (Figure 10 *c*) pattern was similar to that of latent heat flux. $E-P$ depicted a net fresh water loss north of 12°N , with two distinct maxima, one in summer (200 mm/month) and the other in winter (125 mm/month) monsoons (Figure 10 *d*). Throughout the year, the equatorial region receives excess precipitation over evaporation.

Discussion

The above analysis of the upper thermo-haline structure and the surface meteorological parameters brings out the interesting changes from winter monsoon to inter-monsoon as well as the predominant north-south variations. The thermal structure during April-May showed a thin mixed layer, varying from 20 to 35 m, with SST more than 29°C . The core of the ASHSW, seen below the surface in the north, deepens while spreading towards south. Unlike this, during February-March, the thermo-haline structure showed considerable changes in the upper layers. The most dramatic change takes place

in the mixed layer, which becomes deeper than 90 m in the north, reaching up to 120 m at 17°N , while towards south it is shallower (about 65 m). A weakly stratified upper layer was observed, both along the shelf as well as in the open ocean. The surface meteorological conditions during April-May showed predominantly weak northerly winds south of 17°N , which becomes progressively westerlies and stronger towards the north. This indicated the prevalence of north-east monsoon conditions south of 17°N , and setting in of south-west monsoon north of it (Figure 2 *b*). The increase in SST ($>29.7^\circ\text{C}$) over the air temperature (on an average 28°C) indicated the increasing solar insolation during this period (Figure 9 *a*), making the upper layer of the ocean highly stratified. Under the prevailing weak wind conditions and increasing solar insolation, one expects a rather thin and more or less uniform MLD as observed (Figure 9 *c*). During February-March, the winds were weak throughout, on an average 5 ms^{-1} , and were predominantly north/north-easterly indicating the prevailing winter monsoon conditions (Figure 2 *a*). North of 17°N , the air temperature (Figure 9 *a*) was very low (23°C) and accordingly the SST (Figure 9 *b*) also was

low (25°C). However, south of 17°N, conditions were warmer. Under the weak wind conditions, one would expect shallow MLD, as is the case during April–May. In contrast, the thermal structure showed deep MLD (Figure 9 c), specially north of 17°N in the open ocean. This suggests that the deepening of mixed layer in the north, during winter, is not due to wind but is driven by other atmospheric forcings.

In order to probe this further, we computed the heat and fresh water flux into the atmosphere (E–P) along 64°E. During winter the northern Arabian Sea (especially north of 10°N) experiences a net heat loss (about 140 Wm⁻²). This value is slightly lower than that of Hastenrath and Lamb¹⁰ which showed a net oceanic heat loss about 160 Wm⁻² from October to December. An examination of the climatological values¹⁰ of specific humidity during October (19 g kg⁻¹) and January (about 10 g kg⁻¹) in the northeastern Arabian Sea indicates the prevalence of dry air during winter. The cool dry continental air brought into the northern Arabian Sea by the prevailing north-east trade winds enhances evaporation, as evident from E–P values (Figure 9), leading to surface cooling. We estimated the cooling due to loss of heat to the atmosphere using the formula¹¹ $\Delta T = Q t / CM$ [where Q is the net heat flux to the atmosphere (Wm⁻²), t the time period (seconds) over which the heat flux occurs, C the specific heat of water (4.18×10^3 J kg⁻¹ K⁻¹) and M the mass (kg) of the water cooled], which works out to be about 3°C. Apart from the cooling due to evaporation, the decrease of solar insolation (net short wave radiation) from 220 Wm⁻² during October to about 160 Wm⁻² during January¹⁰ also contributes to the further cooling of the surface waters. Thus, in short the observed reduction in SST (by about 4°C) and deep MLD, in the northern Arabian Sea during winter is forced by a combination of enhanced evaporation and reduction in the solar insolation. In fact, this starts during December and persists till end of February. Accordingly, the northern Arabian Sea, north of 15°N, experiences cooling and densification (Figures 4 and 7). This leads to the formation of ASHSW¹², sinking and convective mixing; and injects nutrients into the surface layers from the

thermocline region, as is evident from the shoaling of 2 µM nitrate contour, which is seen at 30 m at 13°N and surfaced at about 17°N (ref. 13). In the Arabian Sea, nitrate in the surface layers is below detection level except during monsoonal upwelling. The nutrient injection to the upper layers of the water column triggers the primary productivity which was about 807 mg C m⁻² per day⁴. On the basis of coastal zone colour scanner data, Banse and McClain¹⁴ deduced the occurrence of highest pigment concentrations north of 20°N. The data presented here is the first ever observational evidence elucidating the physical forcing that brings about the winter bloom in the northern Arabian Sea.

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Arabian Sea upwelling – A comparison between coastal and open ocean regions

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The response of the eastern Arabian Sea to prevailing winds during an upwelling event, in the peak of southwest monsoon, was studied at both coastal and open ocean environment based on the data collected as a part of the Indian Joint Global Ocean Flux Studies (JGOFS) programme. Analysis of wind measurements indicated active upwelling along the southwest coast of India, which gradually propagates towards north. While the dominant long-shore component of the wind induces upwelling in the south, the cross-shore component is instrumental in modifying the density structure of the surface layer, especially in the north, to produce retarding effect. In open ocean, the wind maximum around 17° N and 64° E indicates the axis of the Findlater Jet. The observed surfacing and deepening of the isotherms on either side of the axis are the signatures of the upwelling and sinking associated with the Findlater Jet.

CLASSICAL explanation of coastal upwelling favours wind-induced divergence caused by Ekman transport of the surface water away from the coast¹. The concept is widely supported by observations^{2,3} as well as by theoretical models⁴⁻⁷. Although comprehensive literature on the coastal upwelling system in the Arabian Sea exists, information on open ocean upwelling is somehow scanty. Bauer *et al.*⁸ observed seasonal occurrence of wind-induced upwelling in the northern Arabian Sea, wherein considerable shoaling of the thermocline occurred under the region of maximum winds (Findlater Jet).

In the present paper we examine both coastal and open ocean upwelling during southwest monsoon season. To this end a cruise was undertaken during the peak of the southwest monsoon season (July–August 1995).

Data and methodology

The present investigation is based on the data collected onboard ORV *Sagar Kanya* from 20th July to 12th August 1995, as a part of the Indian Joint Global Ocean Flux Studies (JGOFS) Programme. A Sea Bird CTD was used to collect temperature–salinity profiles from 42 stations up to a maximum depth of 1000 m (Figure 1). Surface meteorological data were collected at six hourly interval along the track. Climatological

wind and sea surface temperature (SST) derived from Comprehensive Oceanographic and Atmospheric Data Set (COADS)⁹ and mixed layer depth (MLD) from Levitus data¹⁰ were also used for comparison with the *in situ* measurements.

Results and discussion

Wind field

The climatological wind during July indicates strong cross-shore component with positive trends from south to north and relatively weak long-shore component with similar trend, except at 15° N where a steep rise in wind speed is noticed (Figure 2a). The SST and MLD distributions also followed the same trend as the wind. It is hence inferred that an upwelling favourable condition existed along the southern shelf, which was less conspicuous towards north.

In the central Arabian Sea, along 64° E, both the zonal

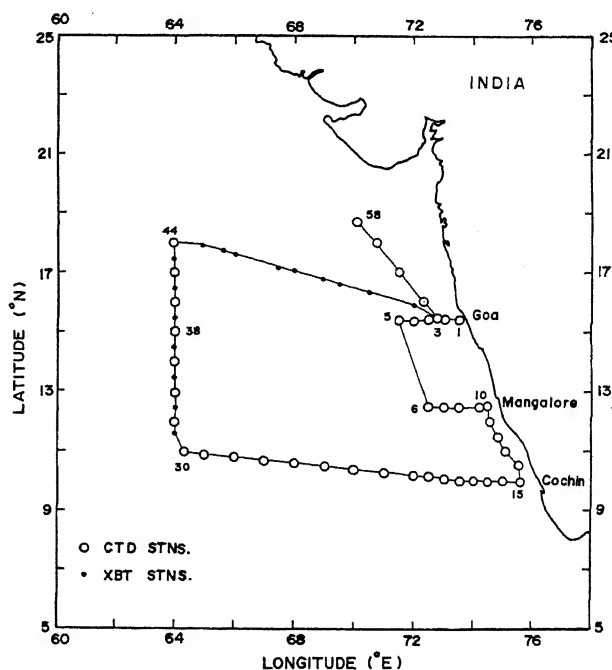


Figure 1. Cruise track and station locations.

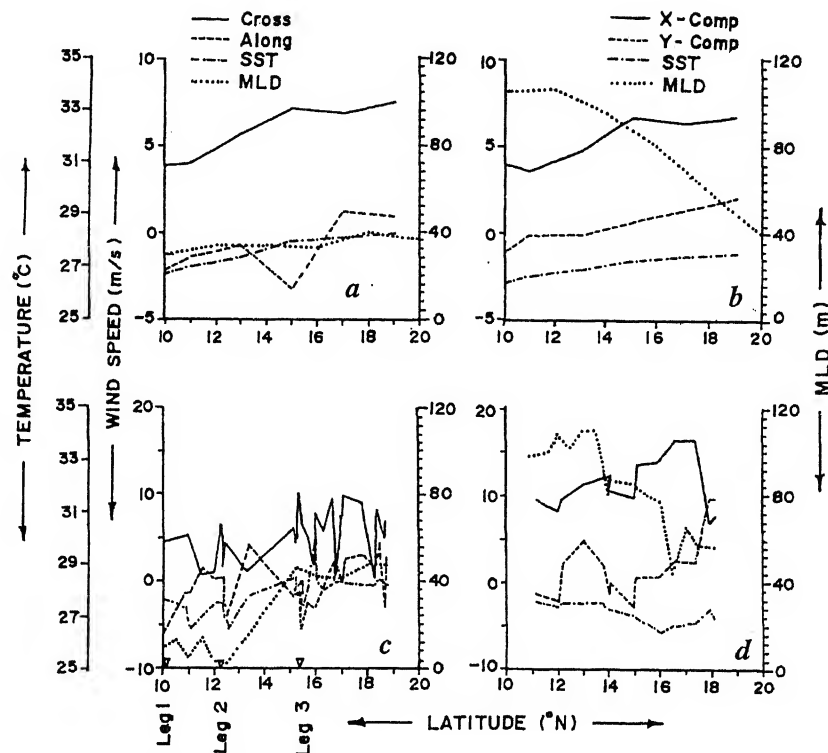


Figure 2 a-d. Variation of zonal and meridional wind component, sea surface temperature (SST) and mixed layer depth (MLD) from 10°N to 20°N along the west coast of India [climatological (a) and in situ (c)] and along 64° E [climatological (b) and in situ (d)] are plotted against latitude during July.

and the meridional components of the wind showed gradual increase from south to north (Figure 2 b). The zonal component was maximum around 17°N and decreased towards north. SST showed a gradual increase of 1°C from south to north. A thick MLD of 110 m was noticed at 10° N, which dropped to 40 m around 20° N. The high zonal component of the wind around 17° N represents the axis of the Findlater Jet. However, the climatological SST distribution does not imply an upwelling at the region of wind maximum.

In order to decipher the upwelling along the coast, *in situ* data were collected at three legs (Figure 1), viz. off Goa (15°N), off Mangalore (12.5°N) and off Cochin (10°N). The observed wind showed a steep rise in both cross-shore (positive towards east) and along-shore (negative towards south) components in all the three legs (Figure 2 c). High eastward wind component (10 ms⁻¹) was observed at leg 1 associated with moderately strong southward component (6 ms⁻¹). At leg 2 (off Mangalore) both eastward and southward components had the same magnitude of 4 ms⁻¹. However at leg 3 (off Cochin) the negative long-shore component (northerly wind) dominates over the positive cross-shore component (westerly wind),

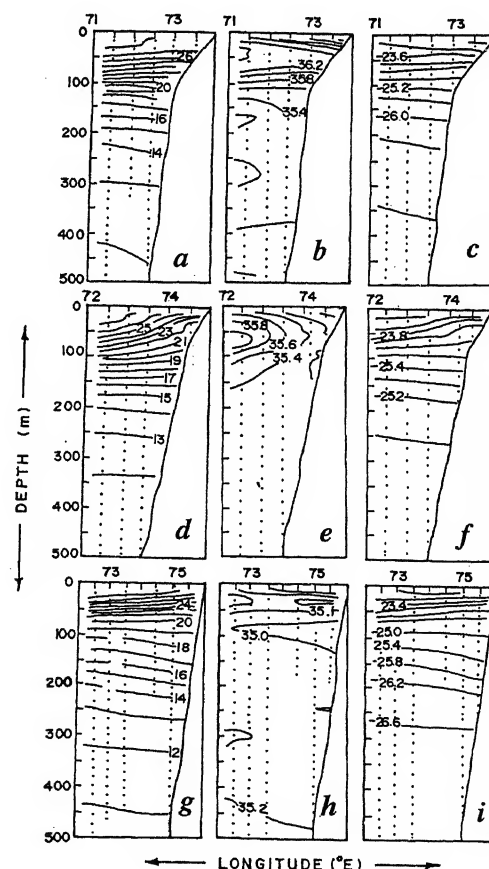


Figure 3 a-i. Vertical distribution of temperature (a, d, g), salinity (b, e, h) and density (c, f, i) along legs 1 (off Goa, top panel), 2 (off Mangalore, middle panel) and 3 (off Cochin, bottom panel).

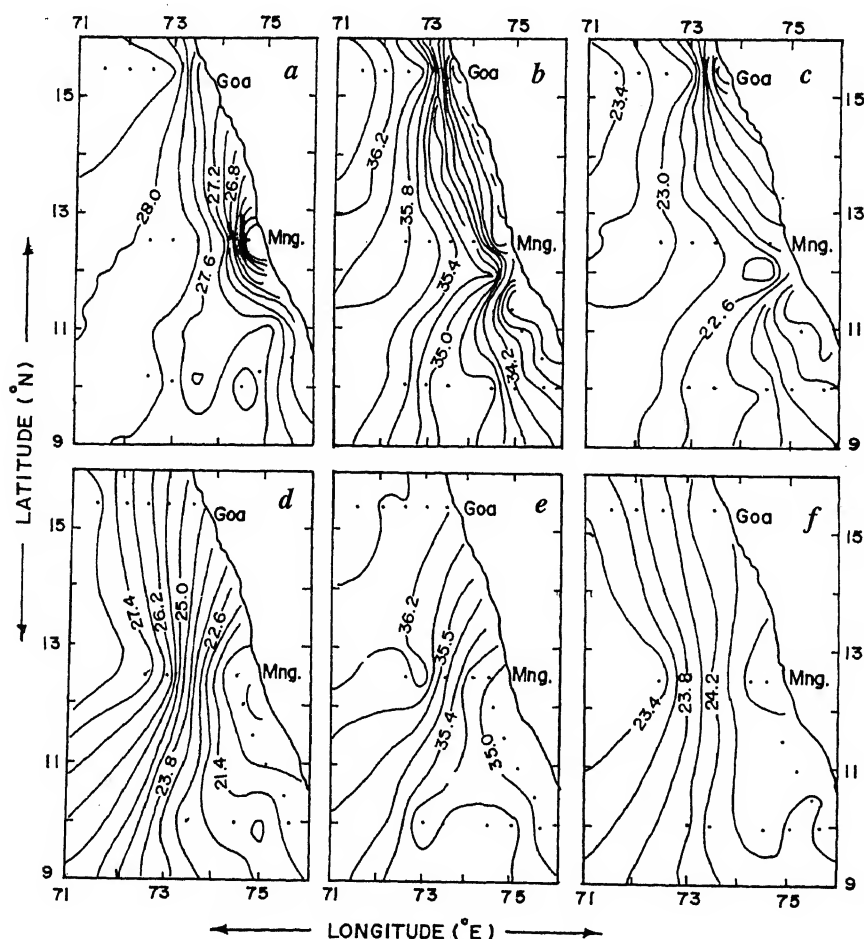
with their magnitudes exceeding 6 and 4 ms^{-1} , respectively (Figure 2 c). The shoaling of the isotherm with low SST off Cochin and Mangalore indicated strong upwelling, and were well correlated with the prevailing wind (Figure 2 c). Off Goa, though the along-shore wind component and the relatively low SST indicated divergence, the high value of MLD does not support the presence of an active upwelling. This is due to strong cross-shore wind present at this latitude, bringing the saline surface water towards the coast which modifies the density field to suppress upwelling. Thus it is obvious that both long-shore as well as cross-shore components play significant role in the dynamics of the underlying water column.

The observed wind and SST along 64°E differ considerably from the climatology, whereas the MLD distribution was consistent. Strong zonal wind component was noticed from 15°N to 18°N with weak meridional component (Figure 2 d). The wind maxima at 17°N (Findlater Jet) coincides with the lowest MLD value of 45 m . The remarkable shoaling of MLD from 100 m at 11°N to 45

m at 17°N was associated with active upwelling and sinking on either side of the wind maximum. This is consistent with the SST distribution where cool water is found at the zone of maximum wind and warm water southward.

Hydrographic field

The vertical thermal structure off Goa (Figure 3 a) exhibits gentle upsloping of isotherms towards the coast producing colder surface water. However, this trend is reversed in the sub-thermocline region and the isotherms were deepening towards the coast. Surface salinities near the coast were almost 0.8 PSU lower than the off-shore values (Figure 3 b). Below 200 m , however, there were no appreciable changes in the salinity. The down sloping of the isopycnals towards the coast indicated the dominance of salinity over temperature in the density field (Figure 3 c). This disappears in the sub-thermocline region where the distribution of density becomes analogous to that of temperature. The isopycnals at the sub-thermocline depth also followed the same



trend as the temperature. This feature was more conspicuous at the 200 m depth.

The nearshore values of SST off Mangalore were much lower ($<23^{\circ}\text{C}$) when compared to the offshore values ($>27^{\circ}\text{C}$) with a gradient of 0.01°C per km (Figure 3 d). The remarkable shoaling of thermocline towards the coast is an indication of the active upwelling which is again reflected in the density distribution (Figure 3 f). Salinity near the coast was as low as 35 PSU (Figure 3 e). Deepening of isotherms and isopycnals at the sub-thermocline depth is noticed at this latitude also.

Although the SST off Cochin did not show any appreciable change, the subsurface isotherms above 100 m did indicate gentle upward sloping towards the coast (Figure 3 g). The deepening of isotherms and isopycnals in the sub-thermocline region is more pronounced at this latitude (Figure 3 g & i). The salinity section, in general, does not have any commendable features (Figure 3 h).

The strong thermal front identified off Mangalore, from the horizontal temperature field, is the manifestation of an

active upwelling system (Figure 4 a). Similar features were not observed off Goa and Cochin. The high salinity tongue near Goa and Mangalore was an indication of on-shore transport of surface water as a result of the prevailing winds (Figure 4 b). The surface density distribution showed the occurrence of lighter water along the shelf and denser water off-shore (Figure 4 c). The thermal gradient at 50 m was three times higher than the surface value with gradient greater than 0.03°C per km (Figure 4 d). The intrusion of high salinity water from the central Arabian Sea, as observed at the surface, was less conspicuous at this depth (Figure 4 e). The lateral density distribution reversed with denser water along the coast and lighter water off-shore (Figure 4 f). The temperature field at 100 m depth is dominated by eddy-like circulation (Figure 5 a). Penetration of high salinity water from north was seen as a well-developed tongue at this depth (Figure 5 b). There was not much lateral variation in density (Figure 5 c). At 200 m, the thermohaline structure was entirely different from that at 100 m (Figure 5 d and e). However, a re-

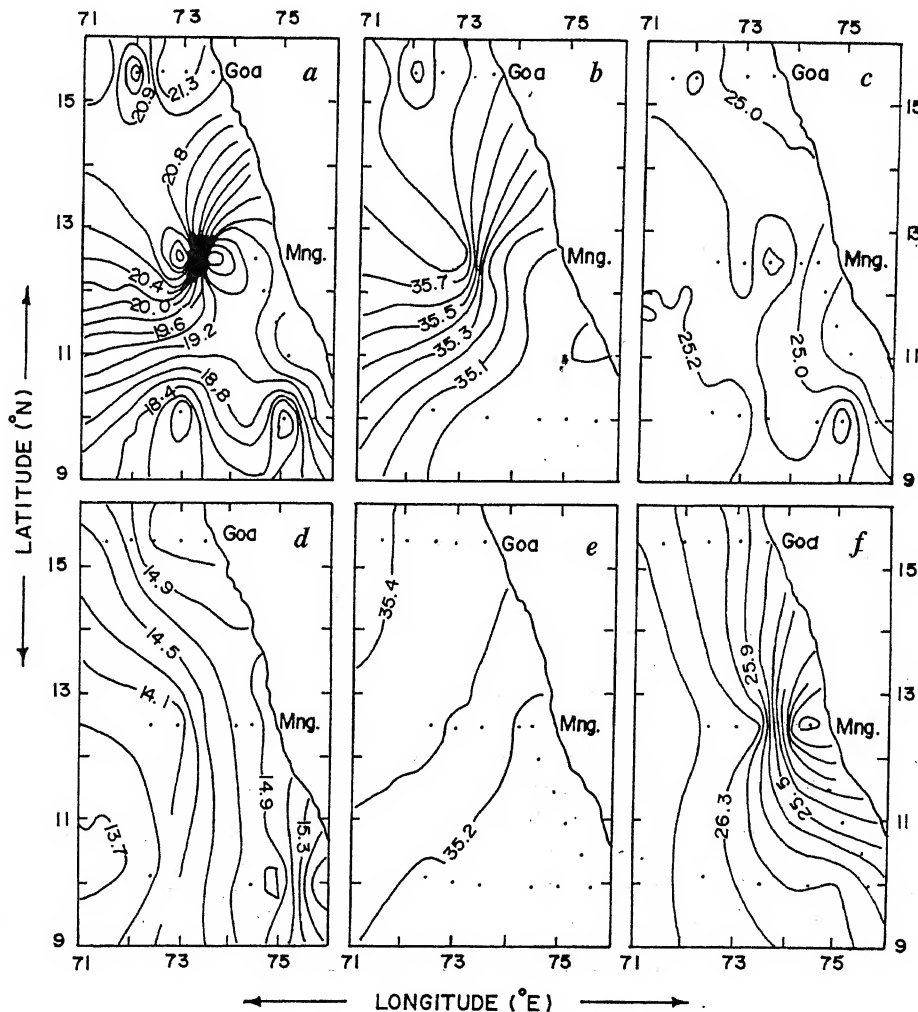


Figure 5 a-f. Horizontal distribution of temperature (a), salinity (b), and density (c) at 100 m and at 200 m (d, e, f, respectively).

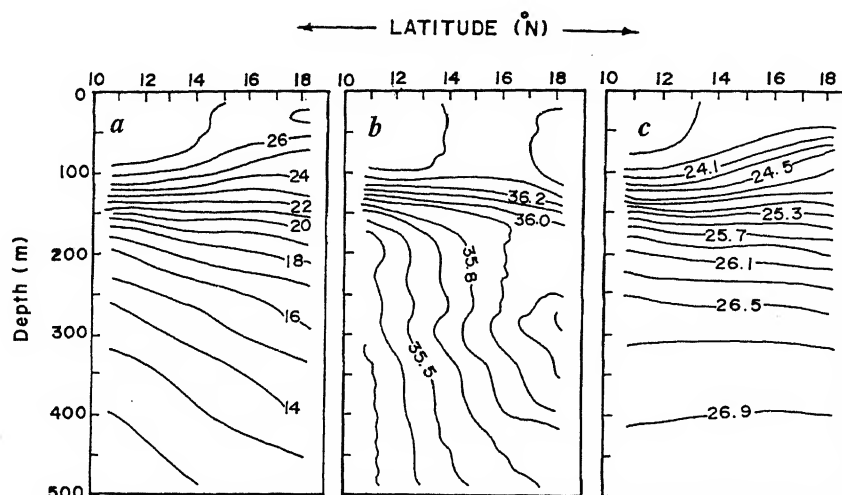


Figure 6. Vertical distribution of temperature (a), salinity (b), and density (c) along 64° E.

versal of lateral density distribution was observed again at this depth with relatively stronger gradient near Mangalore (Figure 5f).

Along 64°E, the shoaling of the isotherms above 150 m was a direct indication of open ocean upwelling north of 14°N (Figure 6a), while the deep MLD (110 m) towards south was associated with sinking. The high salinity core observed at 300 m around 18°N was the Persian Gulf water (PGW) mass, which spreads southward. Below 200 m, both temperature as well as salinity structure showed sinking, resulting in a vertically homogeneous salinity structure (Figure 6b). However, the density structure was analogous to that of temperature in the upper 150 m layer. In the sub-thermocline depth, density structure differed from that of temperature due to the proximity of PGW.

In summary, the wind field along the south west coast of India favoured strong upwelling off Cochin, less strong off Mangalore and relatively weak off Goa. Hydrography also supported this, except off Cochin where data close to the coast were not adequate to make the comparison. The stronger subsurface upwelling front (thermal) in comparison to the surface (the gradient of which was three times the surface value) indicates that the regional dynamics suppresses the surface divergence. A plausible explanation could be sought from the prevailing wind forcing. The cross-shore component of the wind pushes the surface high salinity water (ASHSW) towards the coast which modifies the density field along the shelf and suppresses the Ekman pumping mechanism. In the sub-thermocline depth (200 m) the density distribution indicated the pres-

ence of a coastal undercurrent which is consistent with the earlier studies^{11,12}. The eddy-dominated circulation seen at the bottom of the thermocline (100 m) is the manifestation of frontal instability at the interface between the surface coastal current and the subsurface undercurrent. The open ocean upwelling occurs under the influence of the Findlater Jet, the axis of which was located at 17°N, towards the north while sinking to the south.

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Seasonal variability in oxygen and nutrients in the central and eastern Arabian Sea

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Extensive observations made in the central and eastern Arabian Sea under JGOFS (India) programme suggest strong seasonal variations in concentrations of oxygen and nutrients in the water column. In the intermediate waters (depth range 150–800 m) oxygen concentrations were lowest during winter (with values near zero at ~400 m) in relation to those in the other two seasons (intermonsoon and southwest monsoon). This together with nitrate distributions revealed the occurrence of intense reducing conditions in intermediate waters during winter because of the sluggish water movement and high surface productivity. Secondary nitrite, an indicator of occurrence of denitrification was present at oxygen levels $< 10 \mu\text{M}$. Nitrate deficits reached a maximum of $10 \mu\text{M}$ in winter, whereas, it was half this value in monsoon. The average deficits suggest increasing reducing conditions in the order monsoon ($1.6 \mu\text{M}$), intermonsoon ($3.7 \mu\text{M}$) and winter ($4.0 \mu\text{M}$).

THE Indian Ocean experiences seasonally variable surface circulation. During the southwest monsoon, upwelling occurs along the east coasts of north Africa and Arabia^{1,2} and the southwest coast of India^{3,4}. This results in high biological production, making the Arabian Sea one of the most productive areas in the world oceans⁵. High biological production and subsequent sinking of organic matter leads to high oxygen demand in intermediate waters. Although the renewal of intermediate waters is quite rapid^{1,6}, this high demand leads to the development of intense oxygen minimum at intermediate depths where oxidized nitrogen species are reduced to molecular nitrogen^{6–11}. Denitrification in the Arabian Sea is estimated to be about 1/3 of the global ocean denitrification⁶ which has been shown to vary between northeast (winter) and southwest (summer) monsoon seasons with higher nitrate deficits during the former¹². In this article we report the seasonal variability in reducing conditions in intermediate waters and the influence of physical processes, such as upwelling and winter convection, on the property distributions using the data collected as a part of the JGOFS (India) programme.

Data sets used were collected in the central and eastern Arabian Sea water column during the ORV *Sagar Kanya* cruises SK-91 (April–May), SK-99 (February–March) and SK-104 (July–August) representing the

intermonsoon, winter and southwest monsoon periods, respectively. During the SW monsoon, samples could be collected only up to 18°N . Figure 1 shows the cruise tracks. Water samples were collected using a Seabird CTD rosette, fitted with 12 Niskin bottles of 1.8/12/30 litre capacity. Dissolved oxygen was measured by Winkler titration during SK-91, and by spectrophotometric method¹³ during SK-99 and SK-104. The spectrophotometric measurement of oxygen improved the precision significantly, particularly at low oxygen levels (at $4 \mu\text{M}$ it is $\pm 0.1 \mu\text{M}$). The analyses of nitrite, nitrate and silicate were done using a Skalar Analyser 5100/1. Phosphate was measured spectrophotometrically using the method of Murphy and Riley¹⁴.

The distribution of oxygen in the upper 1000 m during the three seasons is shown in Figure 2. The data show the lowest oxygen concentrations in winter. Its minimum reached near-zero levels during this season while in monsoon it was $\sim 15 \mu\text{M}$. The distribution of nitrate during the three sampling periods is shown in Figure 3. The surface waters were devoid of nitrate during the intermonsoon period. In winter nitrate was about $2 \mu\text{M}$ in the surface waters of northern latitudes, result-

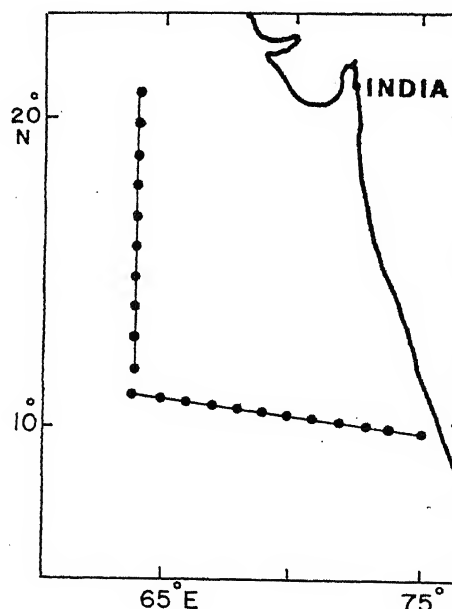


Figure 1. Map showing cruise tracks.

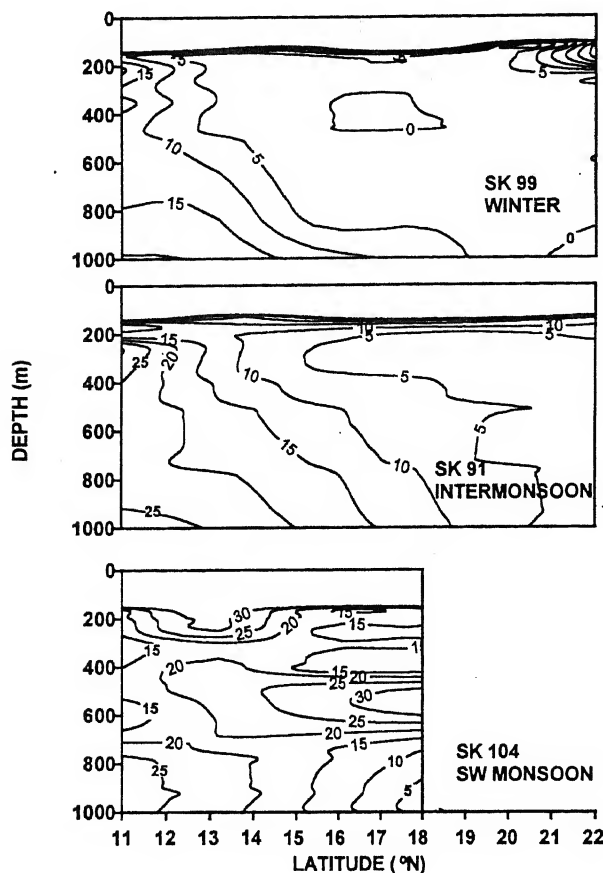


Figure 2. North-south variations in dissolved oxygen (μM) along 64°E ; contours shown for levels $< 30 \mu\text{M}$ only.

ing from wintercooling and convective mixing^{15,16}. These data are consistent with primary productivity measurements during these two seasons^{16,17}. In monsoon there is a conspicuous high nitrate patch in surface waters at about 16°N (Figure 3). This patch is also characterized by lower temperature and higher oxygen concentrations, signatures of upwelling, resulting from gyral circulation¹⁸ or wind regime of Findlater jet¹⁹. The depth profiles of nitrate in general show that, at any given depth the concentrations were lower in the northern latitudes. This probably results from the consumption of nitrate in these regions for combustion of organic matter. Due to enhanced surface productivity in winter, the large amounts of organic matter should be oxidized in intermediate layers, thus leading to oxygen-deficient conditions. When the oxygen is present at trace levels, the bacteria utilize nitrate as oxidant for the decomposition of organic material. During this process nitrate gets reduced to elemental nitrogen as the end product with nitrite as an important intermediate. Thus the presence of nitrite below the thermocline indicates the occurrence and the intensity of denitrification process.

Figure 4 is a plot of nitrite and oxygen in monsoon

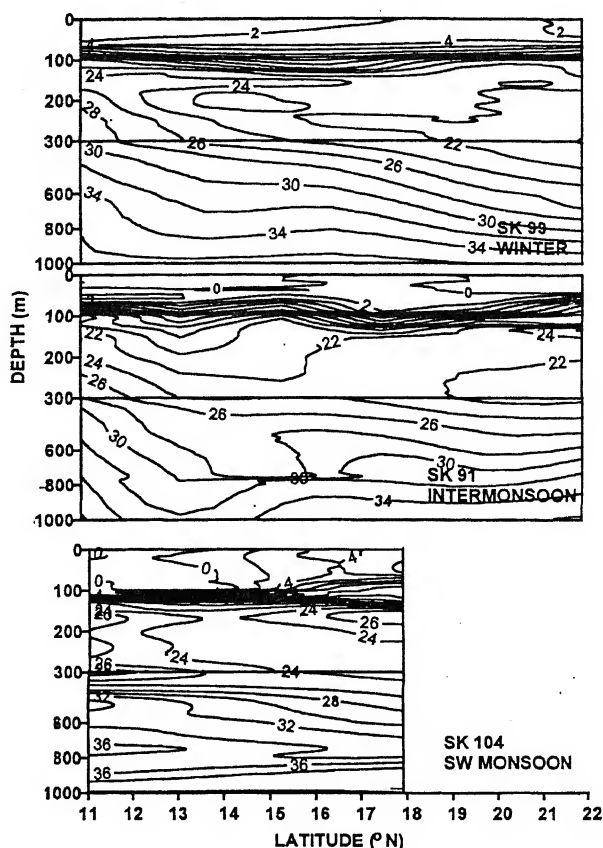


Figure 3. North-south variations in nitrate (μM) along 64°E .

and winter observed in samples from surface to 1000 m. The data show that there are two distinct ranges of oxygen levels in winter where nitrite was present. One is near the upper thermocline region with oxygen concentrations of $175\text{--}275 \mu\text{M}$ and the other is in intermediate depths with oxygen $< 10 \mu\text{M}$. Such distinction could not be made for monsoon since the oxygen in subsurface layers is also relatively high. While nitrite present in sub-oxic waters indicates the occurrence of denitrification, that in the thermocline region originates from nitrification process⁷. Data for monsoon (inset) suggest that secondary nitrite can occur in the presence of trace levels ($5\text{--}10 \mu\text{M}$) of oxygen. However, the oxygen has been reduced further during winter mainly because of its enhanced consumption triggered by the rain of organic matter from surface layers.

The estimated nitrate deficit⁷ (DELN), a measure of nitrate decrease due to its reduction to molecular nitrogen, ranged from 0 to $10 \mu\text{M}$ in the waters between 100 and 1000 m. The highest DELN values were found in winter, particularly in the north (Figure 5) with clearly discernible north-south gradients. In the intermonsoon period, the high DELN values were found at $\sim 14^\circ\text{N}$ be-

cause of relatively low nitrate pocket observed at ~200 m. In monsoon, in spite of the relatively high oxygen in intermediate waters, DELN reached a value

of ~5 μM . This indicates that the Arabian Sea experiences denitrification in all the seasons but its extent is variable. This is obvious from Figure 6 which shows

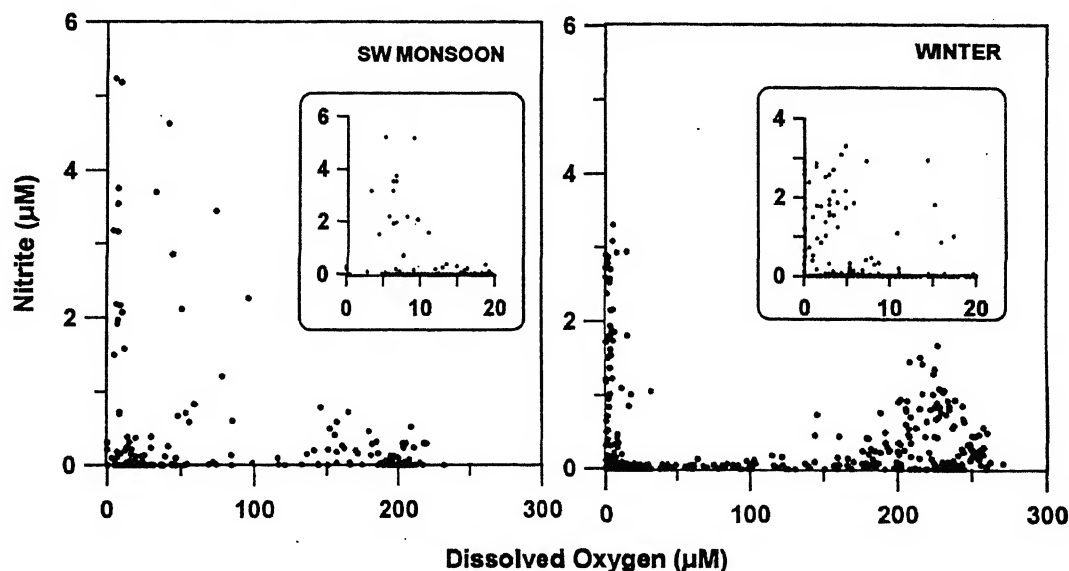


Figure 4. Relation between dissolved oxygen (μM) and nitrite (μM) during monsoon and winter.

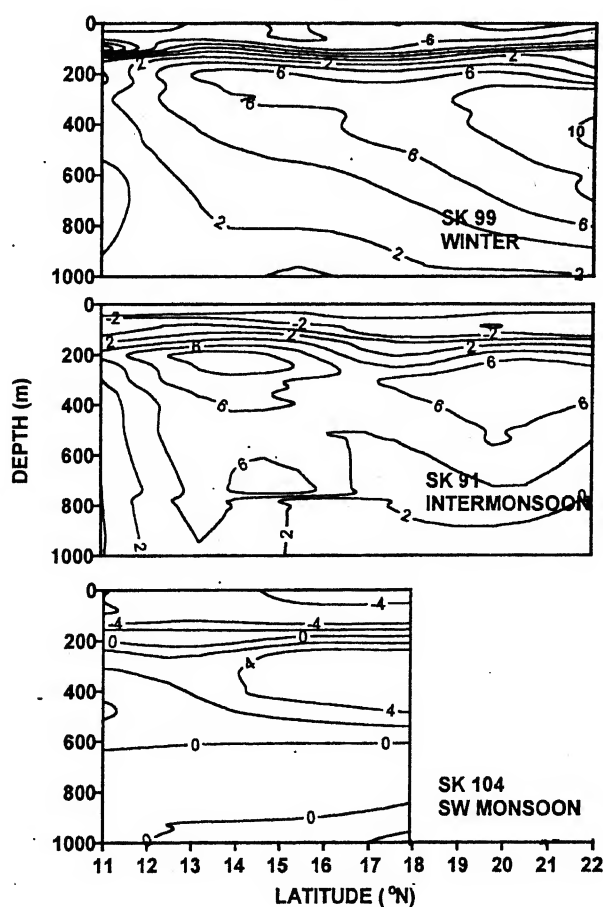


Figure 5. North-south variations in nitrate deficit (DELN, μM), along 64°E.

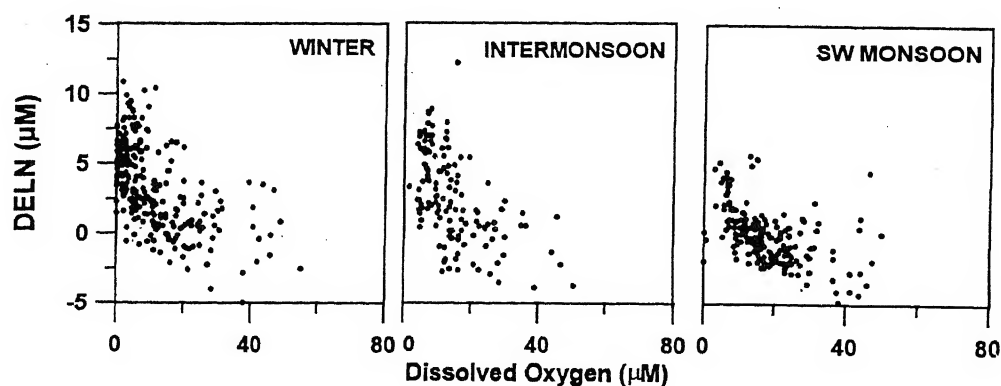


Figure 6. Dissolved oxygen (at $<60 \mu\text{M}$) versus DELN (μM) during different seasons along 64°E .

the relationship between dissolved oxygen and DELN; for O_2 concentration $\leq 60 \mu\text{M}$ for different seasons. A large number of points have positive DELN values in winter particularly below $10 \mu\text{M}$ of oxygen. This is in good agreement with that of nitrite (Figure 4).

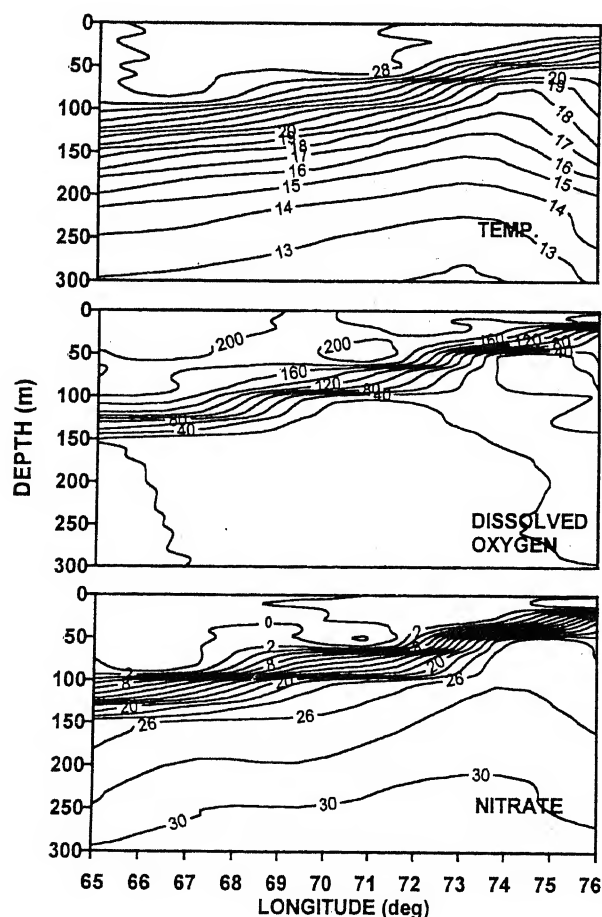


Figure 7. East-west distribution of temperature ($^\circ\text{C}$), oxygen (μM) and nitrate (μM) during SW monsoon.

During winter, convective mixing facilitates the upward pumping of nutrients-laden subsurface layers^{15,16}. Figure 3 shows the influence of this winter mixing on nutrient pumping; the nitrate isoline of $2 \mu\text{M}$ surfaced in the northern latitudes. This process is more vigorous in the northeastern region of the Arabian Sea where the mixed layer deepens to $\sim 100 \text{ m}$, leading to relatively higher biological production. For instance, in winter¹⁷ the primary production was $807 \text{ mgC m}^{-2} \text{ d}^{-1}$ at 21°N and 67°E while it was $335 \text{ mgC m}^{-2} \text{ d}^{-1}$ at 11°N and 64°E about a factor or two higher than that during the intermonsoon season; 310 and $163 \text{ mgC m}^{-2} \text{ d}^{-1}$ at these stations, respectively¹⁷. Hence, the observed variations in nitrate (Figure 3) and total carbon dioxide²⁰ abundances are in agreement with the gradients in productivity. The high surface primary production¹⁷ together with the sluggish renewal of intermediate waters^{1,12} could have resulted in the intense reducing conditions in winter (Figure 5).

Intense upwelling occurs in the northwestern Arabian Sea but of moderate intensity along the southwest coast of India^{4, 21,22}. In addition to that shown in Figure 3, Figure 7 depicts the signatures of upwelling in the eastern side of the east-west section in Figure 1. This occurred to the east of 72°E where the surface temperatures were less than 28°C . The effect could also be

Table 1. Average values of oxygen and nitrate deficit (DELN) in denitrification zone and surface Chlorophyll *a* in the Arabian Sea

Season	Cruise no.	Oxygen (μM)	DELN (μM)	Chl (mg m^{-3})
Intermonsoon	SK 91	17.5	3.72	8.3
Winter	SK 99	10.6	4.02	20
Southwest monsoon	SK 104	18.2	1.62	—

seen in nitrate distribution but not in dissolved oxygen (Figure 7). Increases in nutrients in surface layers due to upwelling result in increased productivity. For example, the primary productivity was $1760 \text{ mgC m}^{-2} \text{ d}^{-1}$ off Mangalore ($12^{\circ}30' \text{N}$, $73^{\circ}30' \text{E}$), $660 \text{ mgC m}^{-2} \text{ d}^{-1}$ near Cochin (10°N , $75^{\circ}35' \text{E}$) and $440 \text{ mgC m}^{-2} \text{ d}^{-1}$ off Bombay¹⁷. This shows a patchiness in surface production. The intensity of upwelling along the southwest coast of India can be suppressed by land run-off^{4,21}. Consequently, variable run-off may have caused the observed patchiness in productivity⁴. However, during intermonsoon it was $199 \text{ mgC m}^{-2} \text{ d}^{-1}$ near Cochin¹⁷ which is considerably less than that in monsoon.

Table 1 shows the seasonal variability in average redox conditions (oxygen and nitrate as indicators) in the Arabian Sea. These are averages for the zone of denitrification, i.e. positive DELN. Results indicate relatively lower dissolved oxygen ($11 \mu\text{M}$) with relatively high DELN ($4 \mu\text{M}$) during winter. This is consistent with the average abundance of chlorophyll *a*. Although the oxygen levels between intermonsoon and monsoon did not differ greatly, the nitrate deficit during the former season was nearly twice that computed for monsoon.

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Seasonal variations in inorganic carbon components in the central and eastern Arabian Sea

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Extensive observations have been made on the carbon dioxide system in the Arabian Sea during three different seasons as part of the Indian Joint Global Ocean Flux Study (JGOFS) Programme. Concentrations of total carbon dioxide and partial pressure of carbon dioxide exhibited seasonal variability, with pronounced north-south gradients in surface layers. Total carbon dioxide in surface waters was higher by $\sim 100 \mu\text{M}$ during winter compared to the intermonsoon period due to winter cooling and convective mixing. The partial pressure of carbon dioxide ($p\text{CO}_2$) in surface layers was generally in excess over the atmospheric value, suggesting that the central and eastern Arabian Sea is a perennial source to atmospheric carbon dioxide. The flux of carbon dioxide to atmosphere reached a maximum of $\sim 40 \text{ mmole m}^{-2} \text{ d}^{-1}$ around 16°N in the central Arabian Sea during monsoon season. The carbon dioxide regenerated from soft tissue was higher during winter and is the least in monsoon. The aragonite saturation horizon occurred around 500 m.

THE northwestern Indian Ocean (Arabian Sea) is a region of negative water balance where evaporation far exceeds precipitation and run-off. Consequently, the upper layers in the region are more saline and weakly stratified¹. This region, strongly affected by seasonal changes associated with monsoon, is one of the most productive areas of the world oceans². The northwestern Arabian Sea is more affected by these changes since intense upwelling occurs in this region during the southwest monsoon season. The northeastern Arabian Sea, on the other hand, experiences winter cooling and convective mixing resulting in the upward transport of nutrients³. These processes make the Arabian Sea important with respect to air-sea exchange of biogenic gases. Carbon dioxide is estimated to be released to the atmosphere at a rate of $74\text{--}79 \text{ Tg C yr}^{-1}$ from this region^{4,6}. Intense oxygen-deficient conditions develop within intermediate layers in the north because of oxidation of large amounts of organic matter that leads to nitrate consumption through denitrification^{7,8}. This results in reducing conditions in the Arabian Sea that could influence the carbonate equilibria and raise the partial pressure of carbon dioxide ($p\text{CO}_2$) in water. Here, we present results of extensive measurements

on carbon dioxide components made as a part of JGOFS (India) programme, in the central and eastern Arabian Sea and discuss their seasonal and spatial variability.

The measurements were made during intermonsoon (SK 91; April to May), winter (SK 99; February to March) and southwest monsoon (SK 104; July to August) seasons on board ORV *Sagar Kanya*. A Seabird CTD rosette system fitted with 1.8/12/30 litre Niskin bottles was used to collect water samples. Sub-samples were drawn into 125 ml stoppered glass bottles taking due care to avoid trapping of air bubbles. All analyses were completed within 24 hours of the collection. Oxygen analyses were done by Winkler's titration during SK 91 while it was done by spectrophotometry on SK 99 and SK 104. Total carbon dioxide (TCO_2) was measured using a Coulometer (Model 5011 of UIC Inc., USA) following the procedure detailed elsewhere⁶ but with a semi-automated sample drawing system. The pH was measured by multiwavelength spectrophotometry at 25°C using Cresol red indicator⁹. The measured pH, on free ion scale, was converted to pH on total scale as described in George *et al.*⁶. Ionization constants of carbonic acid were computed using the relations of Goyet and Poisson¹⁰. Analytical precisions for TCO_2 and pH are $\pm 2.0 \mu\text{M}$ and ± 0.002 , respectively, while those for the calculated parameters, $p\text{CO}_2$ and CO_3^{2-} , are $\pm 4.0 \mu\text{atm}$ and $\pm 1.7 \mu\text{M}$, respectively. The accuracy of TCO_2 measurements was checked using the Certified Reference Materials supplied by Dr A. G. Dickson of Scripps Institution of Oceanography, USA, and was found to be 0.2–0.3%. The carbon dioxide fluxes were computed according to Wanninkhof¹¹ using the measured wind speeds.

The surface TCO_2 concentrations were the highest in winter and are comparable in monsoon and intermonsoon (Figure 1). The north-south gradient is clearly seen in the intermonsoon and winter seasons where sampling could be done from 11° to 22°N . The presence/absence of such a trend during monsoon could not be ascertained as sampling was restricted to $11^\circ\text{--}18^\circ\text{N}$. However, during monsoon there was a patch of low TCO_2 in surface waters around 16°N (Figure 1) which may be related to upwelling driven by the Findlater Jet¹² or to a gyral circulation¹³. In intermediate layers the TCO_2 levels were higher at all depths in the north than

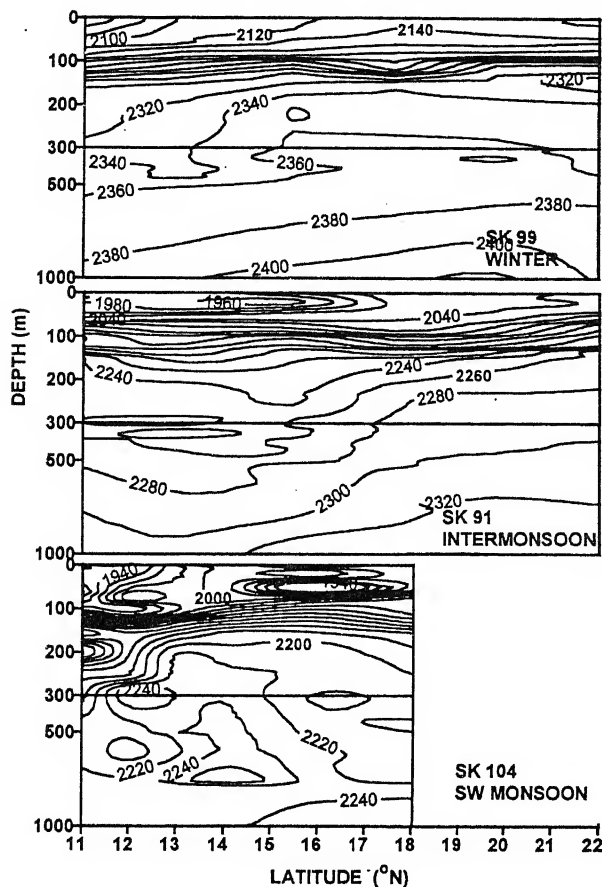
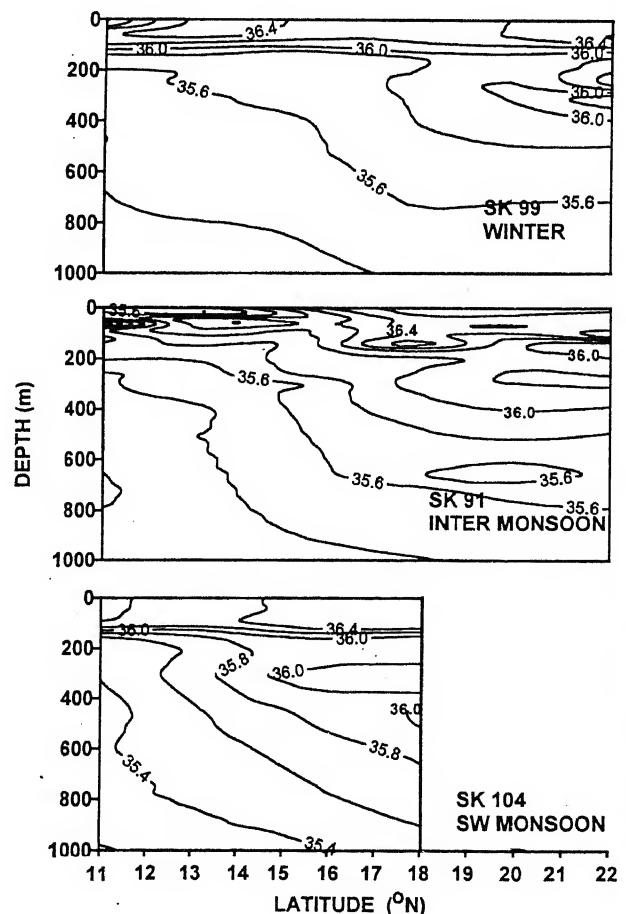


Figure 1. Distribution of TCO_2 (μM) along 64°E during three different seasons winter, intermonsoon and southwest monsoon in the Arabian Sea.

those in the southern Arabian Sea during winter and intermonsoon seasons. This results from a combination of higher biological production, and the consequent regeneration processes and aging of subsurface waters towards the north. The seasonal trends in deep TCO_2 concentrations are consistent with those observed in surface layers. This is because of the fact that the increased surface TCO_2 shall be associated with the supply of nutrients from thermocline region that could enhance production in the upper layers. The subsequent sinking and regeneration of this material in the subsurface layers would lead to proportional increase in TCO_2 . The differences in TCO_2 have, however, decreased from $\sim 100 \mu\text{M}$ in the upper layers to $\sim 80 \mu\text{M}$ around 1000 m between winter and intermonsoon. The seasonal gradients in TCO_2 agreed well with the nitrate distribution shown by de Sousa *et al.*¹⁴. The nitrate concentrations were less than expected from the general trends and consequently nitrate deficit was significant in winter than that in intermonsoon. This larger nitrate deficit is caused by enhanced decomposition of sinking organic material, following higher surface production¹⁵ in winter. The salinity distribution in intermediate layers (Figure 2) indicated increased influence of Persian Gulf Water (PGW),

farther south, in southwest monsoon compared to other seasons. This could be due to seasonal variations in the distribution of outflowed PGW in the Arabian Sea. For instance, the wind fields suggest that the PGW outflow might be carried along the western Arabian Sea in winter but along the eastern part in monsoon (Dr S. Prasanna Kumar, pers. comm.). The PGW is relatively depleted in TCO_2 with $\sim 2150 \mu\text{M}$ (ref. 16) compared to that in intermediate waters of even the south Arabian Sea. Hence, comparatively low TCO_2 was observed in monsoon in intermediate layers of the Arabian Sea due to dilution by PGW. This is augmented by Figure 3, where PGW (rich in oxygen with $\sim 190 \mu\text{M}$) leads to relatively higher oxygen concentrations ($\sim 50 \mu\text{M}$) in monsoon compared to those in winter in subsurface waters of the Arabian Sea.

The higher average TCO_2 ($>2120 \mu\text{M}$) levels in surface waters north of 16°N , during winter, can be attributed to winter cooling and convective overturning³. On the other hand, relatively higher TCO_2 at shallower depths also occurred in waters off the southwest Indian coast during monsoon. This is due to upwelling induced by the prevailing winds^{12,17} that results in the shoaling of 27°C isotherm from 100 m at 64°E to about 10 m at



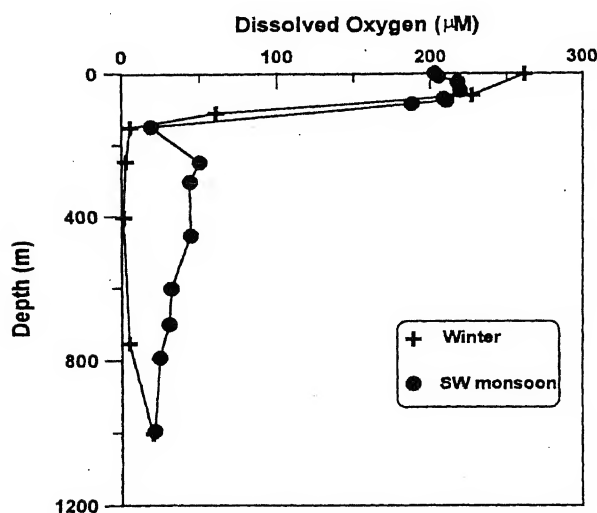


Figure 3. Variation in oxygen between winter (SK 99) and monsoon (SK 104) at 16° N.

76° E (Figure 4). Similarly, isolines of pH (8.00) and TCO_2 (2000 μM) became progressively shallow towards the east which also is the case with pCO_2 (Figure 4). However, there was a large variation in surface TCO_2 along this section that parallels the eastward decrease in salinity, due to the influx of fresh water with lower TCO_2 from land.

Relatively low pH values are observed in the northern Arabian Sea than elsewhere in the North Indian Ocean due to prevailing reducing conditions in the

former region⁶. The depth distribution of pCO_2 (calculated from the measured TCO_2 and pH) is shown in Figure 5 that essentially conform with pH trends. The pCO_2 values in surface waters were generally higher than that in the atmosphere; they mostly centered around ~420 μatm in winter, but with a range of 360–420 μatm during the other two sampling periods. This is again a result of high surface production in winter that subsequently leads to higher pCO_2 levels in subsurface layers. The relatively higher subsurface pCO_2 seems to have been transported into the surface effectively by winter convection. The pCO_2 increased to 1100 ± 100 μatm in intermediate waters (200–1000 m) with discernible seasonal variations (Figure 5). In general pCO_2 in the intermediate waters was higher during winter compared to other two seasons. A patch of high pCO_2 surface water was observed during the monsoon at ~16°N. This results from the offshore upwelling and also from the build up of carbon dioxide partial pressure in intermediate layers in the north. This differential gradient of pCO_2 between subsurface and surface layers drives relatively more carbon dioxide into the surface layers in the north in general. The calculated average carbon dioxide fluxes from sea to air around 21°N and 11°N were about 13 and 0.21 $\text{mmol m}^{-2} \text{d}^{-1}$ in intermonsoon and 1.2 and 0.3 $\text{mmol m}^{-2} \text{d}^{-1}$ during winter, respectively. In monsoon, the flux was about 40 $\text{mmol m}^{-2} \text{d}^{-1}$ around 16°N in the Arabian Sea, where a gyral upwelling was noticed, while it was ~8 $\text{mmol m}^{-2} \text{d}^{-1}$ in the south. Although the fluxes during intermonsoon were as expected from the north-south gradient in regeneration intensities those in win-

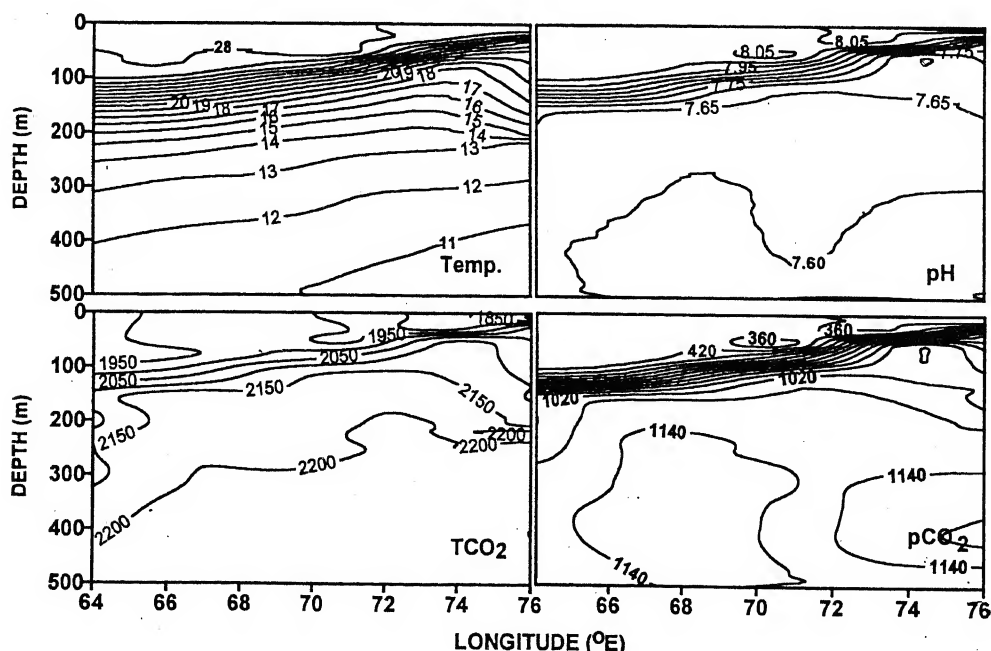


Figure 4. Distribution of temperature (°C), pH, TCO_2 (μM) and pCO_2 (μatm) along an east-west section around 10–11° N.

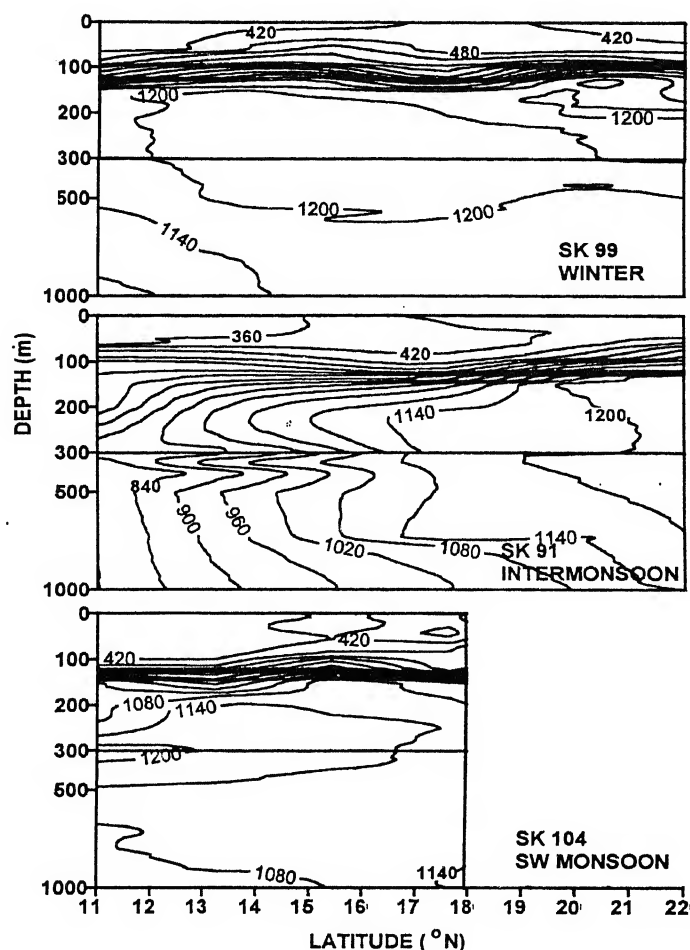


Figure 5. Distribution of $p\text{CO}_2$ (μatm) along 64°E during three different seasons: winter, intermonsoon and southwest monsoon in the Arabian Sea.

ter were not proportional since the evaluated fluxes depend on prevailing wind speeds as well. We observed higher north-south gradients in wind speeds in intermonsoon than in winter. Our results thus reveal that the central and eastern Arabian Sea serves as a perennial source of CO_2 to the atmosphere.

Regenerated carbon (Figure 6) was also found to behave similar to that of TCO_2 (Figure 1). During winter it is higher by $70\text{--}80\ \mu\text{M}$ than in intermonsoon and by more than $100\ \mu\text{M}$ compared to that in monsoon. The regenerated carbon dioxide was evaluated, following the generalizations made for global oceans¹⁸, from

$$\text{TCO}_2(\text{n}) = (\text{TCO}_2 * 35.000)/S$$

$$\text{TCO}_2(\text{pre})(\text{n}) = 2233 - 10.36 * \theta$$

$$\text{TCO}_2(\text{reg}) = \text{TCO}_2(\text{n}) - \text{TCO}_2(\text{pre})(\text{n}),$$

where suffixes 'n', 'pre' and 'reg' indicate normalized, predicted and regenerated components, respectively. θ is the potential temperature. The regeneration amounted to a CO_2 release of $140\ \text{mM}$ at $200\text{--}300\ \text{m}$ in intermonsoon whereas it was about $80\ \mu\text{M}$ and $230\ \mu\text{M}$, respectively, during monsoon and winter seasons

(Figure 6).

The regional variability in calcium carbonate saturation was studied with respect to calcite and aragonite. Saturation carbonate ion concentrations were estimated following the equations of Mucci¹⁹ for the effects of temperature and salinity and of Millero²⁰ for the influence of pressure on the solubility products of aragonite and calcite. Surface waters of the northern Indian Ocean have been found to be supersaturated with respect to both calcite and aragonite^{5,6,21,22}. The present computations suggest that a change-over from supersaturation to undersaturation of aragonite occurs around $500\ \text{m}$ while it is $\sim 2000\ \text{m}$ for calcite in the northern Arabian Sea. But the southern Arabian Sea ($\sim 11^\circ \text{N}$) is supersaturated even at $3000\ \text{m}$ with respect to calcite. The present observations support the view that the deep northern Arabian Sea is relatively more corrosive to carbonate skeletal materials than the southern part^{5,6}.

This study thus establishes a strong seasonal variability in CO_2 components in the Arabian Sea. The TCO_2 and $p\text{CO}_2$ are higher in winter and are driven by cooling

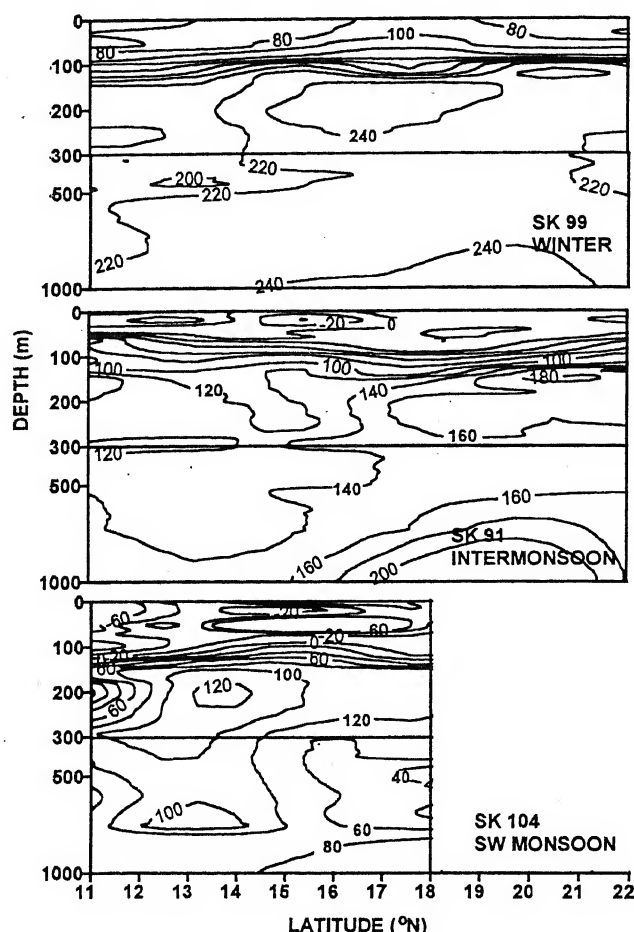


Figure 6. Distribution of regenerated CO_2 (μM) along 64°E during three different seasons winter, intermonsoon and southwest monsoon in the Arabian Sea.

and convective mixing. Intense winds cause larger sea-to-air exchange of CO_2 to atmosphere. The CO_2 regeneration is also intense in winter.

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Phytoplankton production and chlorophyll distribution in the eastern and central Arabian Sea in 1994–1995

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Measurements of primary production, chlorophyll *a*, particulate organic carbon (POC) and nitrogen (PON) were carried out during the inter-monsoon, winter monsoon and summer monsoon seasons of 1994–95 in the central and eastern Arabian Sea. The integrated production rate varied between 193–199, 337–643 and 770 $\text{mgCm}^{-2} \text{d}^{-1}$ respectively in the open ocean (along 64°E) for the three seasons. The corresponding values in near coastal stations were 281–306, 200–807 and 440–1760 $\text{mgCm}^{-2} \text{d}^{-1}$. Column chlorophyll *a* values for these seasons were between 8–17, 13–27 and 34–44 mgm^{-2} in the open ocean and 11–12, 10–34 and 16 to 88 mgm^{-2} for near coastal waters. A subsurface chl maximum (SCM) at ~40–60 m was conspicuous during the intermonsoon period. The seasonal variation in productivity was consistent with the circulation patterns and associated nutrient levels. Phytoplankton and zooplankton (as carbon) contributed 10–22% of POC. Estimated division rate of phytoplankton varied from 0.7 to 3 for different seasons and locations.

INFORMATION on the primary production and chlorophyll is available to a reasonable extent from the northern regions of the Arabian Sea^{1–7}. In recent years renewed interest in the region has been generated as part of the Joint Ocean Global Flux Study to assess the role of the oceanic regions as a source or sink of atmospheric CO_2 on a global context. The aim of this work was to obtain the rate of phytoplankton production and biomass in different seasons and examine the spatial variations. Data obtained during three cruises are presented in this article.

Materials and methods

Sampling was carried out onboard ORV *Sagar Kanya* during April–May 1994 (inter-monsoon), February–March 1995 (winter monsoon) and July–August 1995 (summer monsoon) (Figure 1). Water samples were collected from eight depths (0, 10, 20 m and then every 20 m up to 120 m) using 12 litre Go FLO samplers attached to the plastic coated winch wire. Water samples

from each depth were transferred to 5 Nalgene PC bottles⁸ of 300 ml capacity. 185 kbq of radioactive carbon (^{14}C) in 1 ml of aqueous solution was added to each of the PC bottles (^{14}C was obtained from BRIT, Department of Atomic Energy). To determine the initial activity in the bottles, 0.2 ml from one of the bottles was transferred to a scintillation vial and 0.2 ml of ethanolamine was added to it. 100 ml from one of the bottles was filtered on to 25 mm GF/F (nominal pore size $0.7 \mu\text{m}$) filter paper for determining the initial adsorption of the ^{14}C by the particles in the bottle. From the remaining four bottles from each depth, one was covered with aluminium foil and transferred to a black bag to determine the dark production. Thus, one dark and 3 light bottles were used from each depth for *in situ* in-

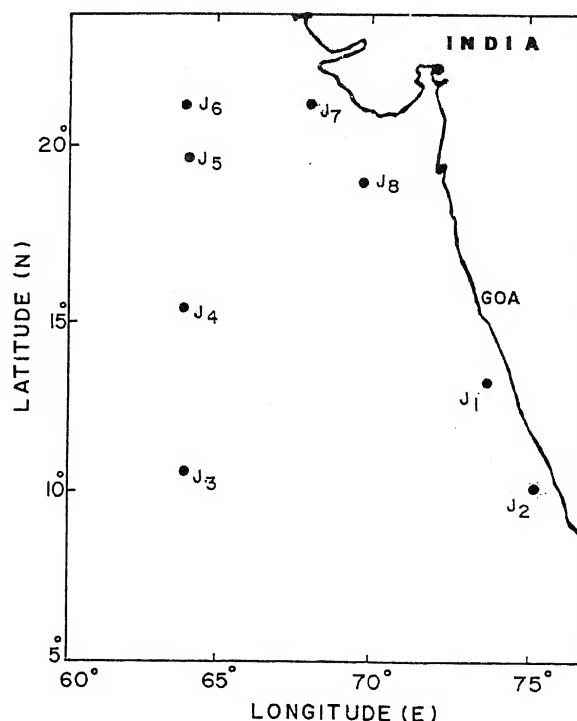


Figure 1. Location of sampling stations.

Table 1. Primary production and chlorophyll *a* in the eastern and central Arabian Sea (1994–95)

Station	Primary production ($\text{mgCm}^{-2}\text{d}^{-1}$)						Chlorophyll <i>a</i> (mgm^{-3})					
	Surface			Column			Surface			Column		
	I	II	III	I	II	III	I	II	III	I	II	III
Open ocean stations												
J3	0.7	3.8	12.8	199	337	770	0.04	0.06	0.42	9	13	44
J4	0.8	12	–	193	606	–	0.05	0.41	0.45	17	27	34
J5	–	6.7	–	–	477	–	0.03	0.27	–	9	21	–
J6	11.9	35.8	–	–	643	–	0.04	0.17	–	8	19	–
Near coastal stations												
J2	–	1.1	49.9	–	200	660	–	0.03	1.34	–	10	16
J1	3.3	–	9.4	281	–	1760	0.05	–	0.09	11	–	88
J8	–	–	12	–	–	440	–	0.22	0.29	–	17	21
J7	8.8	21.4	–	306	807	–	0.05	0.31	–	12	34	–

Inter-monsoon, winter monsoon and summer monsoon observations are indicated by I, II and III. First 4 stations (J3–J6) are from open ocean (along 64°E) and the rest are from coastal areas.

cubation. The bottles were later suspended at appropriate water depths using polypropylene line attached to a buoy. The system was deployed approximately one hour before sunrise, and was retrieved half an hour after sunset. Upon retrieval, the samples were filtered on to GF/F filters and the filters were transferred to scintillation vials to which 0.25 ml of 0.5 N HCl was added and held at room temperature until later analysis. In the shore laboratory sample vials were left uncapped overnight to dry. Liquid scintillation cocktail was added to the vials and after about a day, during which time the scintillation were stabilized, they were counted in a Packard 2500 TR liquid scintillation system. The counted rates were converted to daily production rates ($\text{mgCm}^{-3}\text{d}^{-1}$). The production rates obtained from the triplicates generally agreed within $\pm 10\%$ and averaged to obtain mean values for a given depth (Table 1). Dark bottle production rate was subtracted from the mean rate of light bottle to correct for non-photoautotrophic carbon fixation or adsorption. The daily production rate of various depths was used to calculate the water column integrated production ($\text{mgCm}^{-2}\text{d}^{-1}$).

Chlorophyll *a* was determined by filtering one litre of water samples from each depth using 47 mm GF/F filters (nominal pore size 0.7 μm) under low vacuum (less than 100 mm Hg). The chlorophyll was extracted using 10 ml of 90% acetone (AR) in the dark for 24 hours in a refrigerator. Samples are brought to room temperature and the fluorescence was measured in a Turner Designs Fluorometer before and after acidification with two drops of (1.2N HCl) acid⁸. The chlorophyll *a* was calcu-

lated from the fluorescence using the appropriate calibration factor. The value from each depth was integrated to obtain the column concentration. The instrument was calibrated with pure Chl *a* (Sigma) before the cruise.

For the measurements of suspended particulate organic carbon (POC) and nitrogen (PON), 3 litres of water samples from each depth was filtered on to precombusted 47 mm GF/F filters (nominal pore size 0.7 μm). The filter is removed, wrapped in precombusted aluminium foil and stored frozen in a deep freezer (-20°C) before analysis⁸. Later filters were dried overnight at 60°C . The dried filters were exposed to fuming HCl to remove inorganic carbonate. The filters dried again at 60°C were divided equally into four sections and analysed for POC and PON samples on a Perkin Elmer 2400 CHN analyser. Standards and blanks were run before analysis of samples.

Results

During the inter-monsoon period the surface primary production varied from 0.7 to $11.9 \text{ mgCm}^{-3}\text{d}^{-1}$ in the three open ocean station samples and from 3.3 to $8.8 \text{ mgCm}^{-3}\text{d}^{-1}$ in the two coastal stations (Table 1, Figure 2). The surface production at the northernmost station J6 was higher by an order of magnitude than those of the other two stations. Most of the stations sampled had higher production in subsurface layers compared to that at the surface (Figure 2). Column production for two

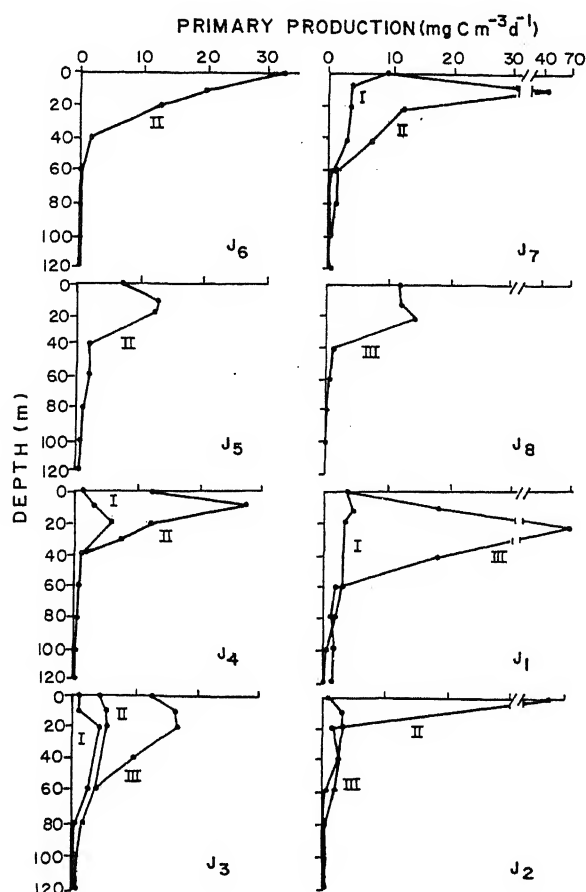


Figure 2. Primary production during the inter-monsoon (I), winter monsoon (II) and summer monsoon (III) periods.

open ocean stations varied from 193 to 199 $\text{mgCm}^{-2} \text{d}^{-1}$ and for the coastal stations from 281 to 306 $\text{mgCm}^{-2} \text{d}^{-1}$ (Table 1). During the winter months the off-shore and inshore surface production varied from 3.8 to 35.8 and 1.1 to 21.4 $\text{mgCm}^{-3} \text{d}^{-1}$. The column production was between 337 and 643 $\text{mgCm}^{-3} \text{d}^{-1}$ in the off-shore region and between 200 and 807 $\text{mgCm}^{-3} \text{d}^{-1}$ in the coastal region. Comparison of production between inter-monsoon and winter monsoon seasons shows higher values during the latter. During the summer monsoon the surface production in the single offshore station was 12.8 while that at the coastal stations varied from 9.4 to 49.9 $\text{mgCm}^{-3} \text{d}^{-1}$. The corresponding column production was 770 at the offshore station while that in coastal stations varied from 440 to 1760 $\text{mgCm}^{-2} \text{d}^{-1}$. Thus during the three seasons sampled, higher production rates were generally observed at the coastal stations with highest being during summer monsoon.

Chlorophyll distribution

The surface chlorophyll concentration was generally

lowest during inter-monsoon seasons, with values in the range of 0.03 to 0.05 mgm^{-3} in both coastal and open ocean stations (Table 1, Figure 3). The column values were also similar ($\sim 11 \text{ mgm}^{-2}$) in these two regions during the season. The chlorophyll distribution exhibited pronounced subsurface maximum (SCM) at 60 m (Figure 3) in all stations sampled. During winter monsoon the surface Chl *a* exhibited a larger range and was between 0.06 and 0.41 and 0.03 to 0.31 mgm^{-3} respectively in the open and coastal water stations respectively. The corresponding column values were between 13 and 27 and 10 and 34 mgm^{-2} . SCM was present only at few stations and was between 40 and 60 m. At two open ocean stations occupied during summer monsoon, surface and column Chl *a* were between 0.41 and 0.45 mgm^{-3} and 34 and 44 mgm^{-2} respectively. Concentrations at the coastal stations varied from 0.09 to 1.34 mgm^{-3} while column values ranged from 16 to 88 mgm^{-2} . At the coastal station J1 where there were evidences of strong upwelling, column Chl *a* and primary production were the highest among all samples measured.

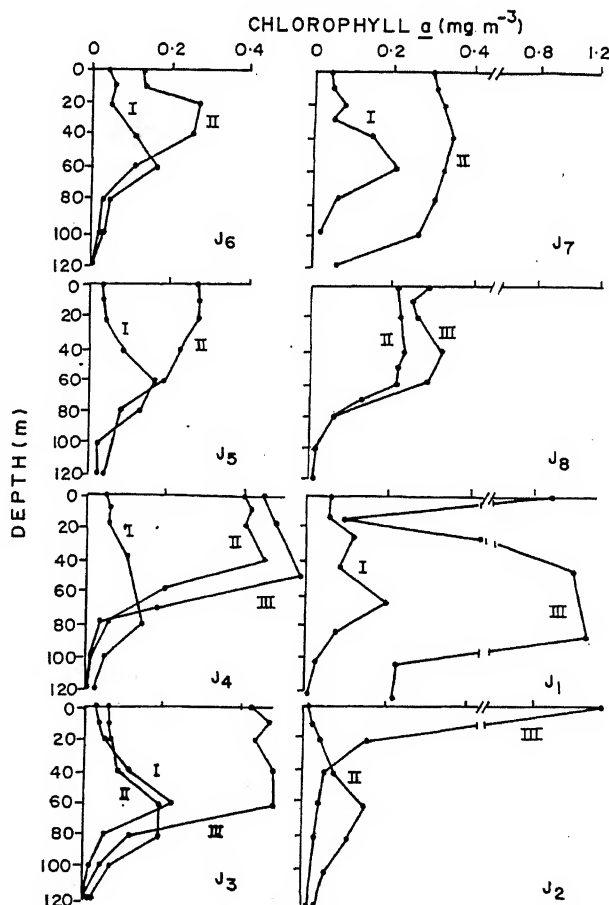


Figure 3. Chlorophyll *a* during the inter-monsoon (I), winter monsoon (II) and summer monsoon (III) periods.

Estimates of the turnover time scales of phytoplankton carbon in the eastern and central Arabian Sea can be derived from the column primary production rate and the standing stock of phytoplankton carbon. The latter was estimated from the measured Chl *a* using appropriate conversion factors (for regimes where nitrate was depleted a factor of 30 was used and where nitrate was repleted and light was limited a factor of 50 was used)⁹. The turnover time scales in the open and coastal stations during the three seasons sampled centered around (1 ± 0.5) days. This would correspond to a division rate of about 0.6–2 per day. These estimates indicate that there is no significant difference in the division rates spatially and temporally in the eastern Arabian Sea.

Particulate organic carbon and nitrogen

Depthwise values of Chl *a*, POC, PON and C/N (atomic ratios) from station J3 are shown in Figure 4 for three seasons. For other stations, measurements were not made for all seasons and therefore not presented here. During inter-monsoon, winter and summer monsoons the POC in the top 120 m had a range from 73 to 150, 140 to 290, 80 to 195 mgm^{-3} , and PON from 7 to 27, 12 to 24 and 5 to 15 mgm^{-3} . C/N ratios were between 7 and 12 up to a depth of 100 m. The ratio was 16 at 120 m.

During winter monsoon C/N ratio was found to be higher with minimum and maximum being 9 and 23. A value of 23 for C/N was found during summer monsoon.

An estimate of the contribution of phytoplankton and zooplankton to the POC could be made by converting the Chl *a* concentration and zooplankton biomass to carbon by appropriate conversion factors. The phytoplankton carbon was estimated by multiplying Chl *a* by a factor of 30 and 50 depending on whether nutrient depleted or repleted and light limited. Zooplankton biomass was converted to carbon¹⁰ by multiplying wet weight by a factor of 0.0235. Contribution by these two was in the range of 5–12 and 5–10%. During monsoon the combined contribution by phytoplankton and zooplankton to POC was 22%.

Productivity in relation to mixed layer settings

During inter-monsoon period, mixed layer along the western shelf was 25 m in the south and 10 m in the north^{11–12}. Along 64°E mixed layer depth (MLD) remained thin with shallowing towards north. During winter monsoon mixed layer varied from 80 m to 120 m, the lower value being at 10°N. Along 64°E MLD deepened from 65 to 125 m between 11 and 17°N and thereafter shoaled to 80 m at 20°N. During summer monsoon MLD in the coastal waters was shallower (15 m) in the

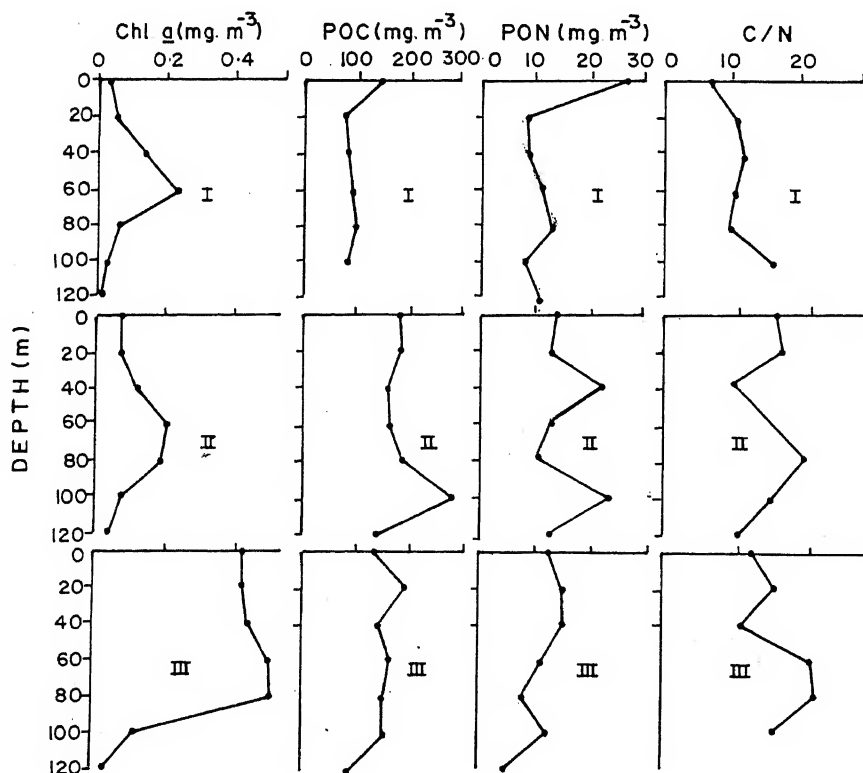


Figure 4. Chlorophyll *a*, particulate organic carbon (POC), particulate organic nitrogen (PON) and C/N ratio (atomic) during inter-monsoon (I), winter monsoon (II) and summer monsoon (III) periods at station J3.

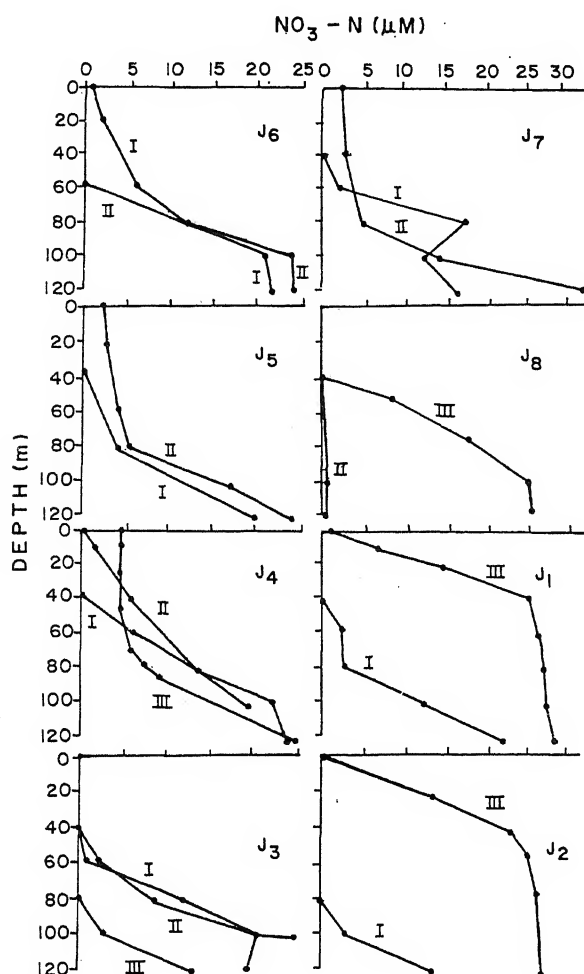


Figure 5. Vertical distribution of nitrate during the inter-monsoon (I), winter monsoon (II) and summer monsoon (III) periods.

south while it deepened northward (45 m). Along 64°E MLD was 90–80 between 11–14°N and about 45 m at 16–18°N.

During inter-monsoon the concentration of nitrate in the shelf and along 64°E was near zero in the upper 40–60 m (Figure 5). 1 μM of nitrate concentration was generally found around 60 m or deeper in most areas. During winter monsoon, the nitrate distribution in the surface waters showed distinct difference between north and south. Shelf waters had near-zero nitrate up to 40 m in the southern region whereas in the northern (off Gujarat) they had 2 μM nitrate at the surface. A similar trend was observed along 64°E. The upper 40 m had near zero nitrate in the southernmost station and further northward 2 μM nitrate was measured at 20 m. During summer monsoon, surface nitrate in the shelf region was high from south of Goa. Thus, off Goa 1 μM nitrate was measured at 24 m, off Mangalore 2.8 μM at 10 m and off Cochin 13.2 μM at 22 m. Along 64°E nitrate was near zero to a depth 100 m in the latitudinal range of

11–18°N except for a patch of 4.6 μM at surface at 16°N.

Discussion

The inter-monsoon period showed lowest primary production and chlorophyll concentration (Table 1, Figure 1). Primary production in the eastern Arabian Sea during April–May was reported to be in the range of 161–1590 (av. 607 $\text{mgCm}^{-2}\text{d}^{-1}$) in the offshore areas and 109–2665 (av. 876 $\text{mgCm}^{-2}\text{d}^{-1}$) in the shelf region¹³. Earlier studies during IIOE have shown that during March–October the shelf waters of Arabian Sea have primary production above 500 and along 64°E between 150 and 500 $\text{mgCm}^{-2}\text{d}^{-1}$. During November–April the same region had more variable production both in the shelf and offshore¹, however the overall values were less with larger areas between 10 and 15°N having low values, i.e. < 100 $\text{mgCm}^{-2}\text{d}^{-1}$. Column primary production has been observed to be more than 1000 $\text{mgCm}^{-2}\text{d}^{-1}$ during southwest monsoon and 500 $\text{mgCm}^{-2}\text{d}^{-1}$ during northeast monsoon over most of the northern Arabian Sea⁷. During August 1987 primary production and chlorophyll *a* between 8 and 20°N was reported to be between 365 and 1130 $\text{mgCm}^{-2}\text{d}^{-1}$ and 13.1 to 32.5 mgm^{-2} in the central Arabian Sea¹⁴. Although the methods adopted for the measurement of primary production earlier were different from those used in the present study, the values during different seasons are generally similar, with the inter-monsoon period having the lowest primary production and phytoplankton abundances. This could be attributed to the low or near-zero nitrate level in the upper layers. During winter monsoon, low surface water temperature caused by cool, dry wind leads to vertical convection and mixing. This enhances the nutrient supply to the mixed layer and results in higher phytoplankton production and chlorophyll *a* has been observed^{15,16}. During this study, the highest primary production was observed during the summer monsoon near the coast. This was due to the upwelling contributing to high nitrate levels in the top layers which in turn supported high phytoplankton production and chlorophyll. Thus at station J1 off Mangalore, where strong upwelling signature was evident, the highest column production and chlorophyll were measured. At this station the nitrate level at 10 m was 2.8 μM , indicating a direct influence of nutrients on the production and chlorophyll. The increase or decrease in both biomass and productivity was in part due to the shoaling or deepening of the mixed layer. For inter-monsoon period, Jochem *et al.*¹⁷ reported primary production in the range of 680 to 790 $\text{mgCm}^{-2}\text{d}^{-1}$ and chlorophyll from 28.7 to 35.8 mgm^{-2} at 18°N, 65°E. During September the primary production and chlorophyll in the coastal Arabian Sea was reported to vary from 235 to 511 $\text{mgCm}^{-2}\text{d}^{-1}$ and from 12 to 46 mgm^{-2}

(euphotic zone was considered as 70 m for computing the column chlorophyll from the mean chlorophyll value given in Table 1)¹⁸.

POC and PON values reported in this work at station J3 (up to a depth of 120 m) had maximum values of 150 and 27 mgm⁻³. Jochem *et al.*¹⁷ observed highest values of both POC and PON from one station from the central Arabian Sea higher than the values reported here. These authors found that subsurface maxima of POC coincided with that of Chl *a*, a result which is not observed in this work. C/N (atomic ratio) measured in this study at J3 was more than that reported by Jochem *et al.*¹⁷. The probable reason for a higher C/N ratio could be due to the higher level of detritus (we have not made any estimate of detritus concentration).

Division rates of phytoplankton as estimated from turnover rate did not show significant seasonal variation with values centering around 2 in the open ocean. Banse⁹ estimated division rates of phytoplankton (from the measurements made during March 1963 and mid-May, 1964) for January–May to be between 0.5 and 1.5 d⁻¹, while for October–November, to be 1.5 d⁻¹. Although there are differences in the type of filters used and techniques adopted for measurements of chlorophyll over the years with corrections applied to earlier data, the estimates of division rates do not seem to differ significantly.

Observations made for the three seasons point towards the seasonal fluctuations in the productivity regimes of the eastern and central Arabian Sea. The entire area becomes more or less oligotrophic during the inter-monsoon period. During winter monsoon the northern latitudes (north of 15°N) became more productive due to winter cooling and convective mixing¹⁵. During the summer monsoon primary production increases along the eastern Arabian Sea as a result of upwelling¹⁸.

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Lack of seasonal and geographic variation in mesozooplankton biomass in the Arabian Sea and its structure in the mixed layer

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Mesozooplankton standing stock, abundance and composition were studied during three seasons (summer, winter and inter-monsoon) from coastal and open ocean waters of the central and eastern Arabian Sea in 1994–95. Concentrations were generally higher in the mixed layer compared to deeper strata. A noteworthy feature was that the standing stocks and abundances did not vary significantly between seasons and areas. Vertical migrations appeared to be in a low key. In all seasons herbivores dominated, followed by carnivores. A few species, common in all seasons, accounted the majority of the population. The 'paradox' of the Arabian Sea, that zooplankton biomass remains more or less invariant, despite seasonally varying primary production regimes, could be explained by a microbial loop.

PRIMARY productivity and phytoplankton biomass show definite seasonal patterns in the Arabian Sea¹. They are high during the southwest (summer) monsoon and part of the northeast (winter) monsoon, and are low during the inter-monsoon periods^{1,2}. They also show north-south and east-west variability² depending on physical events such as upwelling or winter cooling. There are many studies on zooplankton abundances and composition from this region³, however, relatively few of them concentrate on seasonal cycles^{4,5}. Seasonality in zooplankton abundances and composition was addressed as a part of the Indian Joint Global Ocean Flux Study (JGOFS) and sampling was done during three cruises conducted from 12 April to 12 May 1994 (inter-monsoon), 3 February to 4 March 1995 (winter) and 20 July to 12 August 1995 (summer). The stations were positioned to sample the coastal waters along the west coast of India and an open-ocean track along 64° E (Figure 1). A large part of the latter, however, could not be sampled in July–August due to heavy weather.

Materials and methods

The stations (Figure 1) were occupied for at least 24 h to

ensure that sampling of zooplankton was conducted around mid-day and mid-night. Samples were collected with a Multiple Plankton Closing Net (Hydro-Bios, mouth area 0.25 m², mesh width 200 µm). Five depths were sampled at open-ocean stations: 1000–500 m, 500–300 m, 300–base of thermocline (BT), BT-top of thermocline (TT) and TT-surface (mixed layer). At the coastal stations, the two shallow depth intervals were invariably sampled, deeper sampling depended on the bottom depth (Figure 2). Plankton samples were filtered, drained of excess water on absorbent paper and added to

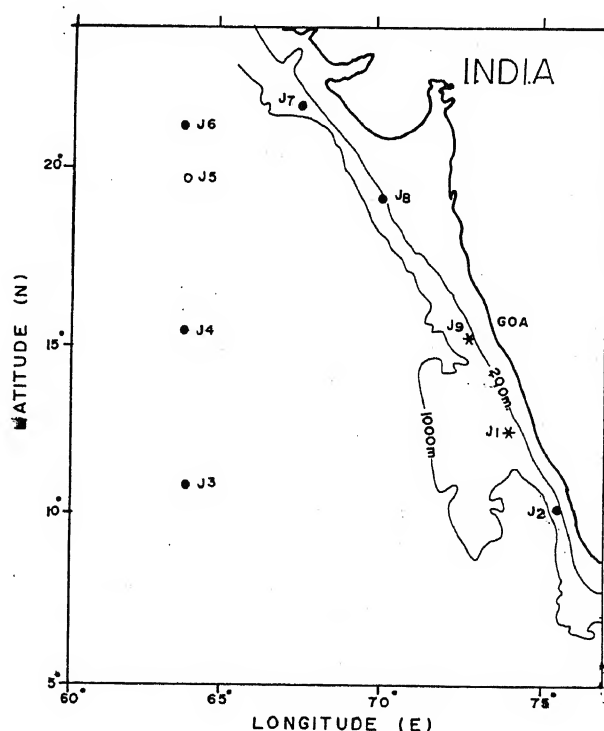


Figure 1. Station locations (filled and open circles and stars) during 3 cruises; open circle (J5)- additional station during February–March, 1995; stars (J1, J9)- additional stations during July–August, 1995; J4 to J7 was not occupied in July–August, 1995; J8 was not occupied during April–May, 1994.

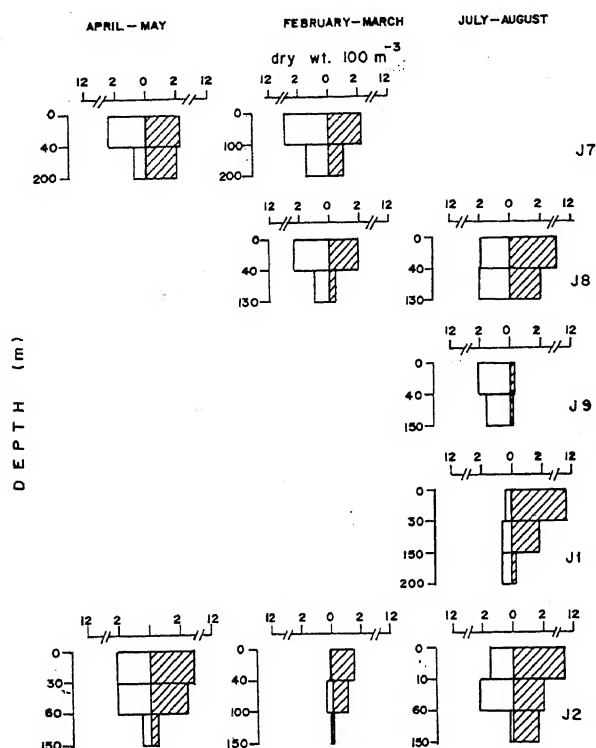


Figure 2. Mesozooplankton biomass (g dry weight 100 m^{-3}) at different depth strata at coastal stations during 3 cruises. Station number is shown. Night-shaded.

known volume of water to estimate biomass. Subsequently, they were preserved in 4% formalin-seawater. A conversion factor of 0.075 g dry weight per 1 ml displacement volume (determined using method described above and without preservation) was used to estimate column and mixed layer standing stocks⁶. Composition of zooplankton from the upper mixed layer, which usually had a higher standing stock, was studied from aliquots. Classification of trophic groups was made according to literature⁶⁻⁹. A Wilcoxon rank test was employed to assess significant spatial (coastal vs open ocean) and day-night variations, if any, in biomass in the mixed layer. This was done separately for each season and then combining data from all cruises. The same test was applied to examine significant day-night variations in total counts and composition of trophic groups of zooplankton in the mixed layer.

Results

Mixed layer was shallow (10–40 m) in April–May and August while it deepened (40–100 m) during February¹⁰. Zooplankton standing stock did not exhibit any definite spatial or temporal trends (Figures 2, 3; Table 1). Aver-

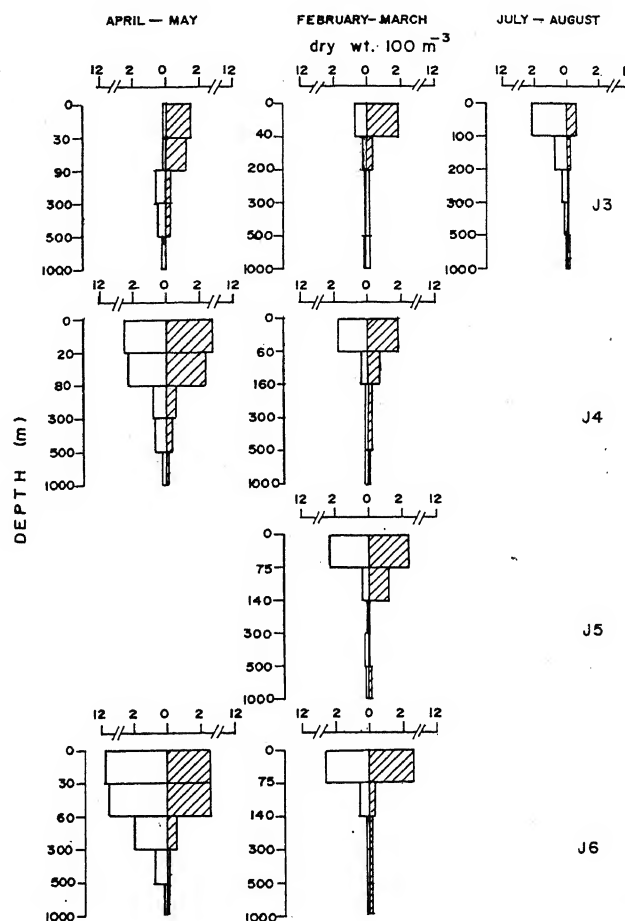


Figure 3. Mesozooplankton biomass (g dry weight 100 m^{-3}) at different depth strata at open ocean stations during 3 cruises. Station number is shown. Night-shaded.

age column standing stocks and the biomass in the mixed layer in coastal and oceanic waters were similar in all three seasons (Table 1, the value for open ocean waters in July–August is based on observation from a single station). The rank test did not show any significant difference ($P > 5\%$) between coastal and oceanic waters within seasons or when all seasons were combined. Biomass decreased drastically with depth, the uppermost two layers sampled accounted for 85 to 95%

Table 1. Average column standing stock of zooplankton (g dry weight m^{-2} , day and night combined, column depths as in Figures 2 and 3) in coastal and open ocean waters during different seasons. Average zooplankton biomass (g dry weight m^{-2}) for mixed layer is given in parentheses

	April-May	February-March	July-August
Coastal	3.7 ± 1.7 (1.4 ± 0.7)	3.1 ± 2.8 (1.6 ± 1.3)	3.3 ± 0.7 (1.3 ± 0.8)
Oceanic	5.7 ± 2.7 (1.5 ± 0.9)	3.1 ± 1.4 (1.7 ± 1.2)	3.3 (1.8)

Table 2. Total number of organisms (per 100 m³, night values) in the mixed layer in coastal and open ocean waters.

	April-May	February-March	July-August
Coastal	51370 ± 22140	4690 ± 29650	61100±50240
Oceanic	91230±59240	81240±32450	23450

in all seasons. Sharp increases in night-time values in the mixed layer were also not clearly discernible ($P > 5\%$) except in some coastal stations indicating that vertical migration was at a low key.

The total number of organisms (night-time values; Table 2) also did not vary much within coastal and oceanic waters in the mixed layer except in August. Neither the

total number of organisms nor trophic groups registered significant night-time increase in the mixed layer. Surprisingly, higher numbers were present in open waters compared to coastal waters during inter-monsoon and winter periods.

Copepods dominated (Table 3), contributing 50 to 88% of the numbers in coastal areas and 77 to 87% in the open ocean. Other dominant groups were Chaetognatha and Tunicata. Herbivores dominated in both areas in all seasons, but appreciable number of carnivores was also present (Table 4). Among copepods, a few herbivorous families, viz. Paracalanidae, Eucalanidae, Calanidae and Clausocalanidae were dominant, accounting for more than 50% of total copepods in all seasons and both areas in the mixed layer (Table 5). These are generally small (< 2 mm, except some species of *Eucalanus*) filter feeding forms. Dominant repre-

Table 3. Relative abundances of major zooplankton groups in the mixed layer (day and night combined, values as a percentage of individuals 100 m³) in coastal and open ocean waters (latter in parentheses)

Group	April-May	February-March	July-August
Hydromedusae*	0.6 (0.1)	0.3 (0.4)	0.002 (-)
Siphonophora*	2.2 (0.3)	0.9 (0.9)	0.04 (0.3)
Ctenophora*	0.1 (-)	- (-)	- (-)
Polychaeta*	1.2 (0.1)	0.3 (0.4)	0.1 (0.3)
Cladocera	0.3 (0.1)	- (-)	0.6 (-)
Ostracoda	0.5 (2.2)	0.5 (1.5)	0.6 (2.1)
Amphipoda*	0.7 (0.9)	0.8 (0.7)	0.2 (0.7)
Copepoda	50 (87.3)	79 (82)	88 (77)
Sergestidae	0.2 (0.7)	0.9 (0.3)	1.1 (2.3)
Euphausiacea	0.4 (0.1)	0.4 (0.6)	0.2 (1.3)
Heteropoda*	0.5 (0.2)	0.2 (0.2)	0.1 (-)
Pteropoda	2.1 (0.1)	0.1 (0.1)	0.6 (0.1)
Chaetognatha*	18 (3.0)	10 (4.9)	3.1 (7.1)
Salps	0.5 (0.1)	0.1 (0.6)	0.3 (-)
Dolioids	0.1 (0.2)	0.2 (0.6)	0.2 (0.7)
Copepoda	15 (3.9)	4.5 (5.8)	0.4 (0.2)
Fish eggs	0.3 (0.3)	0.1 (0.2)	1.8 (4.4)
Fish larvae*	0.5 (0.1)	0.2 (0.1)	0.4 (0.3)
Decapod larvae	3.5 (0.3)	0.8 (0.2)	1.5 (2.0)

- Indicates absence

*Groups considered as carnivores in Table 3. The trophic status of copepods was classified according to genera, see text.

Table 4. Percentage of abundances of different trophic groups of zooplankton in the mixed layer (day and night combined) in coastal and open ocean waters (latter in parentheses)

Group	April–May	February–March	July–August
Herbivores	57 (57)	53 (54)	66 (69)
Carnivores	33 (35)	43 (41)	25 (21)
Omnivores	10 (8)	4 (5)	9 (10)

representatives from these families were *Acrocalanus* spp., *Paracalanus* spp., *Eucalanus attenuatus*, *E. mucronatus*, *E. subcrassus*, *Cosmocalanus darwini*, *Undinula vulgaris*, *Canthocalanus pauper* and *Clausocalanus* spp. Common carnivorous copepods were the calanoid *Euchaeta (rimana)*, the cycloipoid *Oithona* spp. and the poecilostomatoids *Oncaea* spp. and *Corycaeus* spp.

The composition of copepods was quite similar in the coastal and open ocean waters except that species like *Acartia amboinensis*, *Calocalanus pavo*, *C. plumulosus*, *Temora turbinata*, *Centropages alcocki*, *C. furcatus* and *Labidocera* spp. were more or less restricted to coastal areas. Few others such as *Pleuromamma indica*, *Lucicutia flavicornis* and *Acartia negligens* were ubiquitous albeit occurring in low numbers.

Discussion

The striking feature with regard to zooplankton standing stock and abundances was a general lack of seasonal and spatial variations. Another noteworthy feature was the maintenance of high biomass offshore in the mixed layer comparable to the coastal stations. Zooplankton standing stocks were highest in the mixed layer and sometimes to the base of the thermocline (Figures 2, 3). Sharp decrease in biomass with depth has been described from many areas⁶. Although the oxygen minimum layer (<10 μ M, ca. 150–1000 m) in the Arabian Sea might affect distributions, the present study shows that standing stocks start decreasing at shallower depths. Relatively higher zooplankton counts at coastal stations in summer probably resulted from increase in phytoplankton biomass through coastal upwelling (*vide infra*). Although there was some shift in the percentage of abundance of some copepod families, particularly during summer (Table 5), the overall species composition remained more or less the same. Absence of vertical migration by a large number of dominant epipelagic

copepods has been reported earlier⁶. However, possible zonal migrations⁹ would not be detected with the present sampling strategy.

International Indian Ocean Expedition (IIOE) data¹¹ from the Arabian Sea show much lesser displacement volumes from open waters (ca. 10–40 ml 200 m³ from the upper 200 m) compared to present observations (usually about 20–90 ml 100 m³ from the mixed layer). IIOE also shows some decrease in biomass from summer to winter in the western Arabian Sea although for open waters the variations were less pronounced. One reason for the lower biomass obtained may be the larger mesh size used during IIOE (300 μ m) which might not have effectively sampled the smaller, dominant forms mentioned earlier. A second reason may be that while mixed layer had maximum standing stock, the conversion factor used for IIOE data is for the upper 200 m which also included deeper strata with lower biomass.

A fairly high proportion of carnivores (Table 3) in the zooplankton composition of the Arabian Sea has been reported earlier and this seems to be a persistent situation^{4,6,12,13}. Longhurst and Pauly⁹ noted that the biomass of predators is about twice that of herbivores and detritivores in the tropics compared to colder oceans. It would be of future interest to study how the classical concept of a time-lag between the development of herbivores to carnivores apply to the tropics.

The general lack of seasonality in mesozooplankton standing stocks in the Arabian Sea despite seasonally varying plant biomass has been noticed in some recent studies^{4,14–16}. Seasonal and spatial variations were observed in most of the other biological parameters measured along with the present data set. This Arabian Sea paradox of maintenance of high zooplankton biomass can be plausibly explained through the following scenario. It was observed that in February–March, nutrient availability in the euphotic zone was substantial¹⁷ in the northern coastal and oceanic waters (north of 15°N) due to winter cooling and convective mixing. This led to an increase in primary production, chlorophyll *a* phytoplankton and picoplankton cell counts during this season (up to 800 mg C m⁻² d⁻¹, 34 mg m⁻², 1320 \times 10² l⁻¹ and 45 \times 10⁶ l⁻¹ respectively)^{2,18,19}. A similar feature was observed in August along southwest coast of India (up to 1070 mg C m⁻² d⁻¹, 87 mg m⁻², 155 \times 10² l⁻¹ and 50 \times 10⁶ l⁻¹ respectively); during this period the nutrients were supplied through coastal upwelling. But, during inter-monsoon the entire study area became oligotrophic (values were up to a maximum of 310 mg C m⁻² d⁻¹, 16 mg m⁻² and 93 \times 10² l⁻¹, picoplankton was not counted).

Populations of two groups of organisms, viz. bacteria and microzooplankton, however, showed increased abundances during inter-monsoon compared to the other two periods. Highest bacterial counts were 0.09, 0.6 and 2.2 \times 10⁹ l⁻¹ for the winter, summer and inter-monsoon

Table 5. Percentage of abundance of orders and families of copepods (day and night combined) in the mixed layer in coastal oceanic waters (latter in paranthesis)

Family	April-May	February-March	July-August
Order Calanoida			
Calanidae	22 (25.4)	4 (6.3)	10.3 (61)
Eucalanidae	5 (0.5)	6.3 (6.9)	14 (4)
Paracalanidae	17.5 (21.6)	18 (26.8)	24 (11)
Calocalanidae	2.5 (0.1)	1.2 (0.8)	0.1 (0.1)
Clausocalanidae	8 (9)	6.8 (15.7)	9 (4)
Aetideidae	0.4 (0.1)	0.1 (0.1)	5 (0)
Euchaetidae	8.4 (12.7)	12.7 (8)	4 (8.6)
Scolecitrichidae	0.5 (0.1)	0.8 (0.3)	0.2 (0.1)
Temoridae	1.8 (0.1)	0.6 (0.1)	2 (0.1)
Metridinidae	5.7 (0.3)	1.1 (0.5)	1 (2.6)
Centropagidae	1.9 (0.1)	0.2 (0.1)	2 (0)
Lucicutiidae	1 (0.2)	0.6 (0.3)	1 (0.2)
Heterorhabdidae	0.1 (0)	0 (0.1)	0.3 (0)
Augaptilidae	0.4 (0)	0 (0.1)	0.2 (0.0)
Candaciidae	1 (0.4)	0.8 (0.1)	0.3 (0.5)
Pontellidae	3.3 (0.1)	0.4 (0.4)	0.2 (2)
Acartiidae	1.5 (0.1)	1.2 (0.7)	4 (1)
Order Mormonilloida	0 (0)	0 (0.1)	0.2 (0)
Order Cyclopoida	3 (0.6)	12.6 (8)	9 (1.6)
Order Poecilostomatoida	16 (28.5)	32 (23.7)	13 (3)
Order Harpacticoida	0 (0)	0.6 (0.9)	0.2 (0.2)

respectively¹⁹. These were 56 , 139 and $188 \times 10^3 \text{ m}^{-2}$ for microzooplankton²⁰. It could be envisaged that this happened along with a build-up of dissolved organic carbon pool as the winter bloom became senescent and by the microbial loop.

As mentioned earlier, the herbivorous component of the zooplankton consisted mostly of fine filter feeding copepods and tunicates. It is now established that these organisms are capable of feeding on small organisms such as microzooplankton and even bacteria^{21,22}. It would seem that in the Arabian Sea the herbivorous mesozooplankton are able to sustain the biomass during a lean phytoplankton period through a partial switch over from feeding on phytoplankton to the microbial loop. A high zooplankton biomass would in turn support the bacterial population through its metabolic by-products²³. Such adaptations may not be a dominant feature in high latitude food chains since both phytoplankton and zooplankton increase during/after the

spring bloom. Zooplankton populations are low during winter in these latitudes as most of them produce resting eggs to survive this season²⁴. The high zooplankton biomass in the Arabian Sea also apparently sustains a large biomass of mesopelagic myctophid fishes (100 million tons yr^{-1} in northwestern Arabian Sea)²⁵ which migrate to the surface at night to feed exclusively on zooplankton.

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Seasonality and composition of phytoplankton in the Arabian Sea

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Phytoplankton abundance and composition were studied from the central and eastern Arabian Sea during three seasons namely inter-monsoon, winter and summer. Overall, phytoplankton population density was high during winter and summer periods and low during the inter-monsoon. Integrated cell numbers in the upper 120 m was from 0.4 to 1.2, 1.7 to 170 and 0.3 to 10 ($\times 10^8$) cells m^{-2} during inter-monsoon, winter and summer monsoon periods respectively. Diatoms comprised 86% of the total population when three seasons were combined, followed by cyanobacteria (7%) and dinoflagellate (6%). The common diatoms were *Nitzschia* sp., *N. seriata*, *N. longissima*, *Thalassiothrix* spp., *Rhizosolenia* spp. and *Chaetoceros* spp. Some diatoms like *Fragilaria* sp., *Thalassiothrix longissima*, *N. longissima*, *Rhizosolenia* sp. and *Chaetoceros pendulus* were more abundant during the summer monsoon. The genera *Fragilaria*, *Guinardia*, *Hemiaulus*, *Leptocylindrus* and *Lauderia* appeared only during this season. A large number of diatoms were observed in sediment traps during winter.

THE Arabian Sea is an ocean basin where the strength of the physical forcing and biological response vary seasonally. Reversal of surface circulation during monsoon, seasonality in nutrient distribution and high light intensities drive phytoplankton growth processes in the Arabian Sea. Although there is considerable information on the primary production in the Arabian Sea¹⁻⁷, not much is known about phytoplankton abundance and composition except from inshore waters of the west coast of India⁸⁻¹⁰. In this article we compare the phytoplankton communities in the Arabian Sea during inter-monsoon, winter and summer monsoon periods. The research forms part of the Joint Ocean Global Flux Studies. As the main goal of these process studies is to quantify biogeochemical cycling in the Arabian Sea, here we present information on phytoplankton biomass and analyse their seasonal variations from coastal as well as open waters of this region.

Materials and methods

The study was conducted as part of the Joint Global Ocean Flux Studies (JGOFS-India) in the central and eastern Arabian Sea. Collections were made onboard

ORV *Sagar Kanya* in the three cruises conducted from 12 April to 12 May 1994 (inter-monsoon), 3 February to 4 March 1995 (winter monsoon) and 20 July to 12 August 1995 (summer monsoon).

Forty-eight samples were collected from 6 stations during April–May while 56 and 40 samples from 7 and 5 stations, respectively were processed from winter and summer cruises (Figure 1). 250 ml seawater samples were drawn at 0, 10, 20, 40, 60, 80, 100 and 120 m depth from Go-Flo samplers fitted on a CTD rosette. Samples were fixed in 1% Lugol's iodine and preserved in 3% formaldehyde solution. The samples were stored in the dark at low temperature until enumeration within a period of one month after collections.

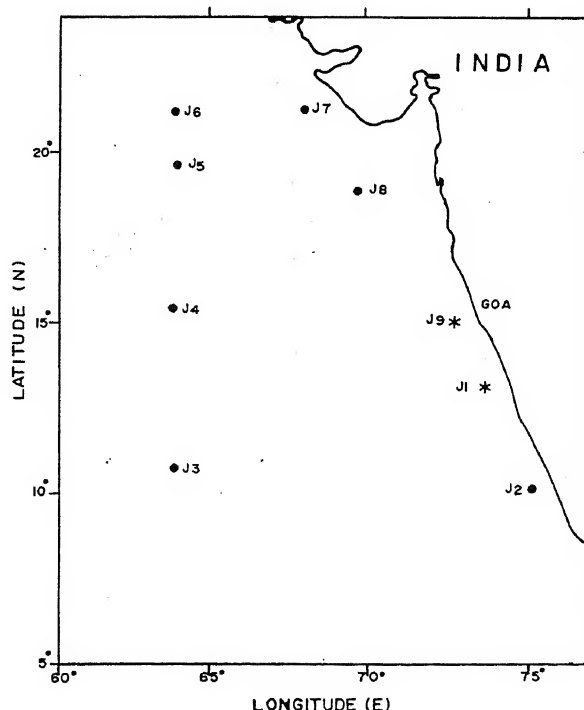


Figure 1. Station location of ORV *Sagar Kanya* cruises: 12 April–12 May (inter-monsoon), 3 February–4 March (winter monsoon), 20 July–12 August (summer monsoon) '●' denotes the stations occupied, '*' denotes additional stations during July–August, 1995, J4–J7 were not occupied during April–May, 1994, J9 and J11 were not occupied during February–March, 1995.

SPECIAL SECTION: JGOFS (INDIA)

A settling and siphoning procedure was followed to obtain 20–25 ml concentrate. 1 ml of this concentrated sample was examined microscopically in triplicate under a stereoscopic binocular microscope (magnification 100×) in a Sedgewick–Rafter Plankton Counting Chamber for phytoplankton of size >5 µm. Species identification was done according to Subrahmanyam¹¹ and Wimpenny¹². Chain-forming cells were counted on a per cell basis and empty cells were excluded. Phytoplankton not identified to species was collectively placed under generic listings.

Results

Abundances

The integrated phytoplankton cell counts for upper 120 m for different seasons are presented in Table 1. The abundances were generally higher during the winter monsoon, in northern areas both in coastal and open ocean stations compared to inter and summer monsoon periods. The highest cell count (133×10^3 cells l⁻¹) was observed during winter at 15°N 64°E, at 20 m depth (Figure 2 a). Phytoplankton population was dominated by *Nitzschia* sp. (47%) at this depth. Diatoms formed the major group in this season making an overall contribution of 87%, whereas, dinoflagellates contributed 7% followed by cyanobacteria comprising *Trichodesmium* sp. (6%).

The abundance of phytoplankton was quite low during the inter-monsoon period. Integrated cell counts ranged from 0.4 to 1.4 (10^8 cells m⁻²). The offshore stations at 15°N 64°E had the maximum cell count (9.4×10^3 cells l⁻¹) at 20 m (Figure 2 a), where *Nitzschia seriata* and *Chaetoceros* sp. were the dominant phytoplanktons contributing 50% and 15% respectively. Diatoms formed 90% of the phytoplankton during this season followed by cyanobacteria (6%) and dinoflagellates (4%).

The only open ocean station (11°N 64°E) sampled

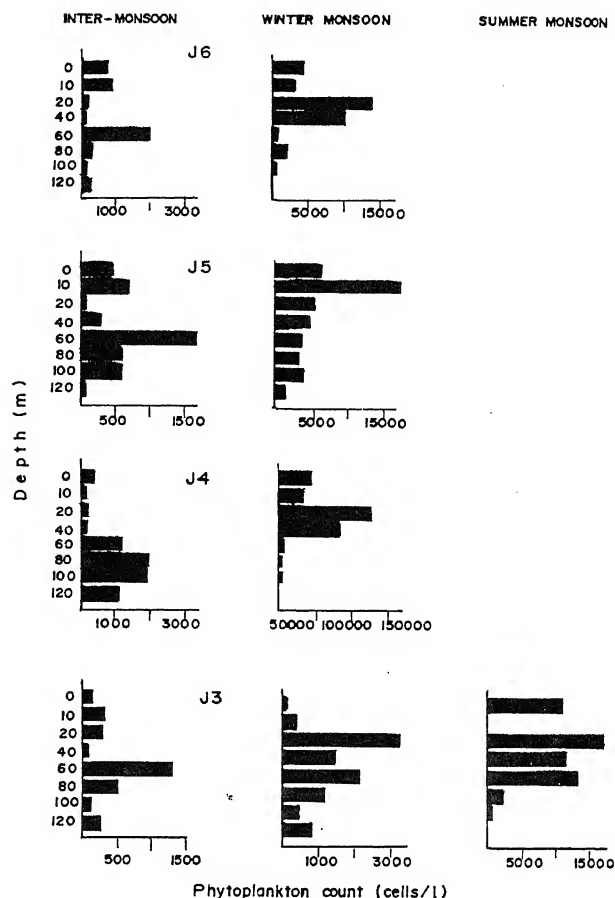


Figure 2 a. Cell concentrations (l⁻¹) at various depths at offshore stations during different seasons.

during summer monsoon had the highest cell count of the season (Table 1). This was also relatively high comparing the three seasons at this southernmost station. The highest cell count (15.5×10^3 cells l⁻¹) was recorded at 20 m depth (Figure 2 a), where diatoms *Thalassiothrix longissima* (29%) and *Chaetoceros pendulus* (26%) formed bulk of the population. Comparable density (13.9×10^3 cells l⁻¹) was recorded at 0 m depth at the coastal station J1 where active upwelling was observed¹³, but numbers rapidly decreased with depth. Unlike at the open waters, the common diatoms recorded were *Rhizosolenia stolterfothii* (18%) and *Guinardia flaccida* (13%). The distribution of phytoplankton at coastal stations during the summer monsoon was restricted to a depth of about 80 m (Figure 2 b). However at coastal station J2, comparatively higher phytoplankton count (2000 cell l⁻¹) was seen at the depth of 100 m (Figure 2 b). The population at this depth was entirely dominated by *Fragilaria oceanica* comprising 96% of the cell count. Overall, diatoms contributed 89% followed by dinoflagellates (6%) and cyanobacteria population decreased to 1% during this season.

Table 1. Integrated phytoplankton abundance (10^8 cells m⁻²; 0–120 m depth) at different stations and seasons

Stations	April–May	February–March	July–August
Open stations			
J3 11°N 64°E	0.5	1.7	10
J4 15°N 64°E	1.2	47.0	—
J5 19°N 64°E	0.8	6.1	—
J6 21°N 64°E	0.7	55.4	—
Coastal stations			
J7 21°N 67°E	0.4	170.0	—
J8 19°N 70°E	—	91.0	0.4
J9 15°N 70°E	—	—	0.3
J1 12°N 74°E	—	—	2.3
J2 10°N 75°E	1.4	3.4	0.7

— indicates no observation was made.

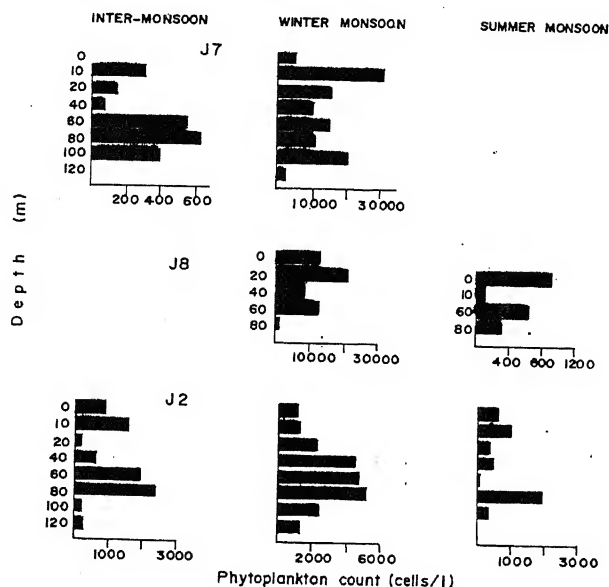


Figure 2b. Cell concentrations (l^{-1}) at various depths at coastal stations during different seasons.

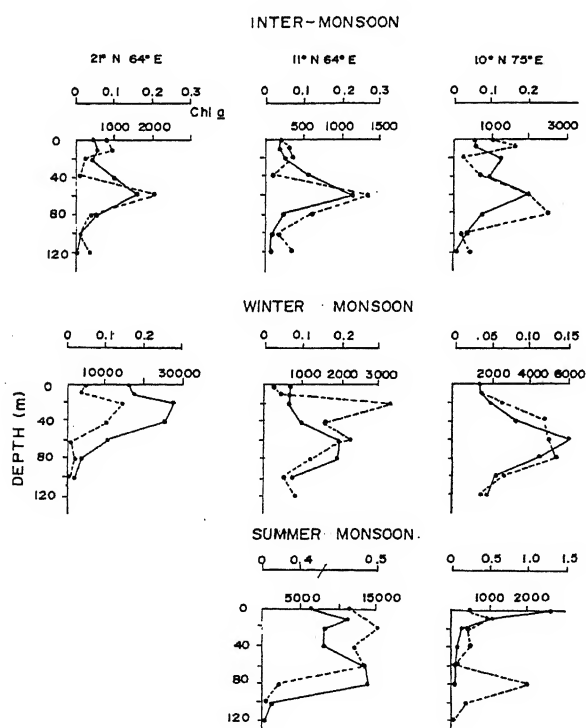


Figure 3. Phytoplankton distribution ($cells\ l^{-1}$, solid lines) and chlorophyll *a* ($mg\ m^{-3}$, broken lines) at 3 stations during the inter-monsoon and winter monsoon seasons and 2 stations during the summer monsoon.

Higher phytoplankton population was observed at subsurface depths (10–80 m) at the different stations during the three seasons (Figure 2a, b). Generally, the peak in phytoplankton abundance showed a direct relation to the subsurface chlorophyll maximum (SCM)

layer which varied from 20 to 60 m (Figure 3).

Assemblages

A variety of phytoplankton taxa (36) were identified. The more abundant genera and species are listed in Table 2. Diatoms were the dominant group followed by dinoflagellates and cyanobacteria. During inter-monsoon, population was dominated by species like *Nitzschia seriata*, *N. closterium* and *N. pungens* at all the stations (ca. 25% of total population), except at $15^{\circ}N\ 64^{\circ}E$, where *Navicula* was common (22%) followed by *Rhizosolenia* spp. (10%) and *Chaetoceros* sp. (9%). Dinoflagellates were represented by *Ceratium* sp. and *Peridinium* sp. constituting 4%.

During winter monsoon, the dominant phytoplankton taxa were *Nitzschia* sp. (37%), *N. seriata* (21%), *Chaetoceros* sp. (8%), *Rhizosolenia* spp. (7%) and *N. longissima* (5%). Dinoflagellates were represented by *Ceratium* spp., *Peridinium* sp., *Dinophysis* sp., *Prorocentrum* sp. and *Gymnodium lunula*. *Rhizosolenia* spp. however, were generally dominant at coastal stations, constituting 20%. Some diatoms like *Bellarochea* sp., *Schroderella* sp., *Cyclotella* sp., *Streptotheca* sp., *Climacodium* sp., and *Hyalodiscus* occurred in few numbers.

Maximum variety of diatom species occurred during summer monsoon. Important species in terms of abundance were *Thalassiothrix longissima* (22%), *Nitzschia longissima* (8%), *Chaetoceros* sp. (5%), *Rhizosolenia* sp. (5%), *R. stolterfothii* (4%), *N. seriata* (4%) and *Thalassiosira* sp. (2%). Species of diatoms belonging to the genera *Ditylum*, *Eucampia*, *Fragilaria*, *Guinardia*, *Gyrosigma*, *Hemiaulus*, *Hyalodiscus*, *Leptocylindrus*, *Lauderia*, *Lymnophora* and *G. flaccida* occurred only during summer monsoon, and at the coastal stations in particular.

The other species which were recorded in low densities in the present study and not listed under Table 2 or elsewhere, were *Actinopterychus* sp., *Asteromphalus* sp., *Biddulphia aurita*, *B. mobiliensis*, *Ceratualina* sp., *Climacosphaenia* sp., *Chaetoceros affinis*, *C. contortum*, *C. schuttii*, *C. holsaticus*, *C. teres*, *C. decipiens*, *C. curvisetus*, *C. constrictus*, *C. didymis*, *Melosira* sp., *Nitzschia bilobata*, *N. directa*, *Pleurosigma* sp., *Planktoniella* sp., *Rhizosolenia delicatula*, *R. hebatata*, *Stephanopyxis* sp., *Thalassiosira* sp., *Thalassiothrix nitzschoides*, *T. frauenfeldii*, *Tropedoneis* sp., *Ceratium furca*, *C. fusus*, and *C. bucephalum*.

Discussion

In this paper we present the floristic documentation of the seasonal composition and abundance of the

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phytoplankton of the eastern and central Arabian Sea.

Species encountered in this study have been reported in the checklist for diatoms of the Indian Ocean¹⁴, and previously recorded from the west coast of India^{8,9}.

The population was dominated by diatoms at both coastal and open waters in all seasons. The abundance of this group (all seasons combined) was 86%. Subrahmanyam and Sarma¹⁰ reported the dominance of diatoms during summer monsoon but list Dinophyceae as more abundant during post-monsoon (September–October) period. However, dominance of diatoms during both post and premonsoon has also been noted in earlier

studies from the coastal waters of west coast of India¹⁵. The most common diatoms encountered in the present study were *Nitzschia* spp., *Navicula* sp., *Chaetoceros* spp. and *Rhizosolenia* spp.

Among the seasons sampled, the winter and summer monsoon were more conducive for phytoplankton population. About 25% of the total photosynthetic production of the Arabian Sea occurs in the northern regions in winter¹ as corroborated in our cell counts (Table 1). During winter monsoon, deepening of the surface layer and vertical mixing due to convection¹³ enhanced nutrient availability, leading to increase in phytoplankton

Table 2. Mean seasonal concentrations (cells l⁻¹) of common phytoplankton species at coastal and open ocean stations (I = intermonsoon, W = winter monsoon and S = summer monsoon)

Phytoplankton species	Open ocean			Coastal		
	I	W	S	I	W	S
Diatoms						
<i>Biddulphia</i> sp.	20	—	—	80	33	—
<i>Cerataulina</i> sp.	—	—	166	—	533	20
<i>Chaetoceros</i> sp.	980	2500	533	200	9900	460
<i>Coscinodiscus</i> sp.	240	75	17	400	350	120
<i>Navicula</i> sp.	1820	3100	—	760	3000	200
<i>Nitzschia</i> sp.	660	49575	166	—	9700	160
<i>N. seriata</i>	2100	27950	650	—	6066	120
<i>N. closterium</i>	400	100	16	640	2666	100
<i>N. longissima</i>	—	8650	800	—	—	640
<i>N. pungens</i>	—	—	—	80	6400	—
<i>Rhizosolenia</i> sp.	620	3850	450	320	7366	440
<i>R. alata</i>	—	2375	—	—	3233	60
<i>R. cylindrus</i>	60	225	—	—	333	—
<i>R. fragillissima</i>	—	75	—	—	1366	140
<i>R. stolterfothii</i>	—	100	50	—	1066	740
<i>R. setigera</i>	—	25	—	—	266	220
<i>R. styliformis</i>	—	150	—	—	266	80
<i>Thalassiosira</i> sp.	140	975	—	40	1066	440
<i>T. nitzchiodes</i>	—	—	—	400	900	220
Dinoflagellates						
<i>Ceratium</i> sp.	220	200	—	40	33	20
<i>Peridinium</i> sp.	20	150	—	—	133	—
Cyanobacterium						
<i>Trichodesmium</i> sp.	—	100	—	480	—	60

population (Table 1, Figure 2 a, b). The northern coastal and offshore areas above 15°N latitude, were found to be more productive during winter¹⁶. Kuz'menko¹⁷ also reported high abundance of phytoplankton in terms of numbers as well as biomass during this season from the central region of the Arabian Sea.

Along the south west coast of India, upwelling which bring nutrients to the euphotic zone, starts with the onset of the summer monsoon in May–June, and intensifies in July–August^{18–21}. This leads to marked increase in phytoplankton growth (e.g. at 12°N 74°E) where active upwelling was observed²². The corresponding column Chl *a* and column primary production values at this station were also high (80 mg m⁻² and 1.7 gCm⁻²d⁻¹ respectively)²². This is also reflected in the surface cell counts we obtained. The only offshore station, at 11°N 64°E, which was sampled during this season also showed higher cell count (Table 1) and high column Chl *a* (44 mgm⁻²) and primary production (770 mgCm⁻²d⁻¹), despite the absence of upwelling signature^{22, 23}. An interesting feature observed during the summer monsoon was the decrease in cyanobacteria population from 6% to 1%. This is probably because *Trichodesmium* requires stable, warm conditions for their growth²⁴. The occurrence of *Fragilaria oceanica* has been noted to be common during the summer monsoon in the coastal waters of the west coast of India²⁴. The phytoplankton production and cell counts during the inter-monsoon remained low. The nutrient supply and chlorophyll values also showed declination during this season^{16, 25}.

We observed that maximum concentrations of phytoplankton usually occurred at intermediate depths (10–80 m). Thus the phytoplankton abundances showed a direct relation with the subsurface chlorophyll maximum layer, at the nutricline²⁴, especially, during the inter-monsoon period (Figure 3). The restriction of their distribution at coastal stations during summer monsoon to a depth of ca. 80 m seems to result from high turbidity.

A large number of diatoms were seen in floating sediment trap samples (deployed during winter) at 300 m depth at 15°N 64°E station (Dr V. Ramaswamy, pers. comm.). The diatoms *Rhizosolenia* sp., *Nitzschia* sp., *Thalassiosira* sp., *Navicula* sp., *Coscinodiscus* sp. common in the water column were found in significant numbers (ca. 3×10^3 cells l⁻¹). Presence of intact diatoms in traps at shallow depth (80 m) has been reported from the western Arabian Sea during inter-monsoon season²⁶. The floral composition also was somewhat similar. The dominant forms were *Rhizosolenia*, *Chaetoceros* and *Guinardia* spp. Although phytoplankton may be consumed or mineralized in the surface waters (or form aggregates and sink out), their presence as recognizable forms at this aphotic depth (300 m) points to role of

phytoplankton in rapid carbon export. This may also indicate that during productive periods (summer and winter), mesozooplankton grazing does not effectively control the phytoplankton production in the Arabian Sea²⁷.

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Studies on the microzooplankton from the central and eastern Arabian Sea

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Numerical abundance and composition of microzooplankton in the upper 200 m were studied from the central and eastern Arabian Sea during three seasons. Protozoans, comprising of ciliates (loricates and aloricates), flagellates and sarcodines were dominant, ranging from 55% to 91%. Among metazoans, nauplii and copepodite stages were common. Microzooplankton abundance was generally higher in the upper 100 m water column during all the three seasons. The column values were from 69,000 m⁻² to 188,000 m⁻² (inter-monsoon), 7350 m⁻² to 56,350 m⁻² (winter monsoon) and 10,800 m⁻² to 139,150 m⁻² (summer monsoon). Seasonal averages were 700 l⁻¹, 130 l⁻¹ and 310 l⁻¹ respectively. A maximum of 5000 l⁻¹ was observed during summer at 5 m at a coastal station. Microzooplankton carbon in three different seasons ranged from a minimum of 4 µg C l⁻¹ during summer monsoon to a maximum of 36 µg C l⁻¹ during inter-monsoon and was higher than that of mesozooplankton. Peaks in population observed during inter-monsoon season, when phytoplankton productivity was low and relatively high bacterial abundance was observed, indicated a microbial loop.

As a part of the Joint Global Ocean Flux Studies (JGOFS-India), abundance and composition of microzooplankton were studied from the central and eastern Arabian Sea. They form a key component in cycling of carbon and other nutrients in marine surface waters^{1,2}. There is virtually no study on microzooplankton from this area. Microzooplankton are defined as the phagotrophic animal forms which pass through a 200 µm mesh netting³. They are taxonomically heterogeneous comprising both protozoa and metazoa. It is known that the microplankton form a significant proportion of the plankton community in the epipelagic zone, in oceanic, coastal and estuarine waters⁴⁻¹³. In India, studies on the microzooplankton are limited to the work on tintinnids from the Vellar estuary, Pichavaram mangroves and the adjacent coastal areas along the east coast¹⁴⁻²⁰. We report here, for the first time, a study on the annual cycle of the microzooplankton abundance and composition from the Arabian Sea.

Materials and methods

Samples for microzooplankton were collected following the JGOFS protocol²¹. Water samples were ob-

tained from a CTD rosette sampler fitted with Go-Flo bottles (12 litre capacity, General Oceanics). The Go-Flo bottles were triggered during upcast at depths of 200, 150, 100, 50 and 5 m.

The collections were made during three seasons: inter-monsoon (April–May 1994), winter monsoon (February–March 1995) and summer monsoon (July–August 1995) onboard O. R. V. *Sagar Kanya* cruises (Figure 1). Samples were processed in the following manner: To quantify the larger (20–200 µm) microzooplankton, 10 litres of water from the Go-Flo bottle was filtered through 200 µm net into a bucket. This water was then slowly passed through a wide area of 20 µm net. The filtration was done carefully and slowly to avoid bursting of delicate forms due to pressure exerted while filtering. The filtered micro-zooplankton was then transferred to 500 ml GF/F filtered sea water and preserved with 1% Acid Lugol's solution, 1% EM hexam-

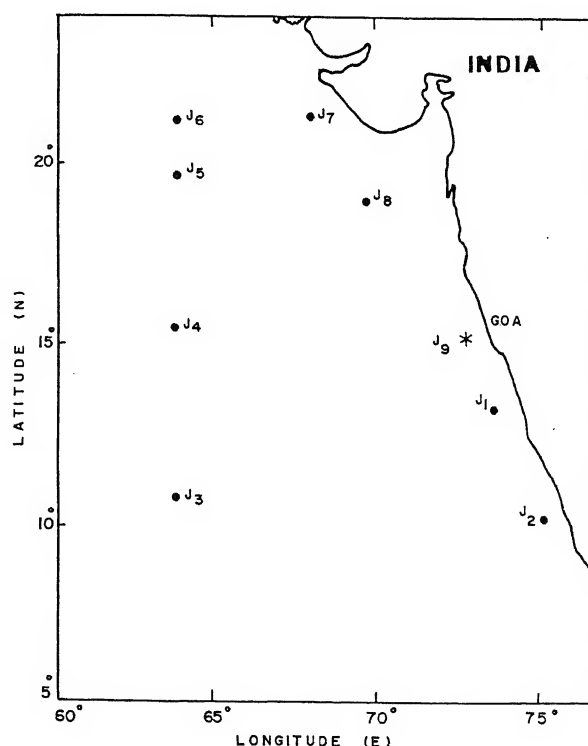


Figure 1. Station locations during three cruises. ●, stations sampled, *, additional station sampled during July–August 1995. Stations J2–J7, J1–J8 and J1–J4, J8, J9 were sampled during April–May, February–March and July–August respectively.

ine buffered formaldehyde and 2 mg/l of strontium sulphate. Samples were refrigerated in dark until analysed later in the laboratory. These samples were used for enumeration, identification and to measure the biomass.

In the laboratory these samples were left undisturbed and allowed to settle for more than 48 h. These were then concentrated to 50 ml by siphoning out the supernatant and observed under an inverted microscope with phase contrast optics⁷. Microzooplankton were identified to genus level based on literature²²⁻²⁶. They were assigned to the following five groups: metazoa, tintinnids, sarcodines, flagellates and aloricates. Metazoa were not counted during intermonsoon and cells were grouped as loricates and other protozoans. Cell dimension (μm) of protozoans was determined from microscopic measurements in order to compute the volumes. This was converted to carbon using a factor of $0.19 \text{ pg C } \mu\text{m}^{-3}$ for ciliates²⁷ and $0.14 \text{ pg C } \mu\text{m}^{-3}$ for dinoflagellates²⁸. A conversion factor of $16 \text{ ng C/individual}$ was used for metazoan microzooplankton²⁹. While computing the biovolume of protozoans 40% cell shrinkage due to preservation³⁰ was taken into consideration. The cell volume of tintinnine ciliates in the present study was assumed to be 50% of lorica volume. Mesozooplankton biomass (0–200 m depth) estimated as displacement volume³¹ was converted to dry weight (1 ml displacement volume = 0.075 g dry wt.) and to carbon (34.2%)³².

For studying the abundance of heterotrophic flagellates, 50 ml of fresh sea water samples from depths mentioned earlier was fixed in 2% glutaraldehyde (only during the winter and summer monsoon). These were then stained with DAPI (final concentration of $5 \mu\text{g/ml}$)^{33,34} and counter-stained with Proflavin and after 5 minutes concentrated onto $0.8 \mu\text{m}$ black Nuclepore filters of 25 mm diameter^{34,35}. These were stored at 5°C until observed under an epifluorescent microscope (Olympus) under UV excitation with a blue filter. Only unbroken well-defined organisms were counted.

Results

Composition

Protozoans were dominant at all stations during the three seasons. They mostly comprised of ciliates (both loricates and aloricates), sarcodines and flagellates. Tintinnids were represented by 30 genera. Among these 11 genera, *Tintinnopsis*, *Eutintinnus*, *Favella*, *Parundella*, *Amphorides*, *Codonellopsis*, *Rhabdonellopsis*, *Dictyocysta*, *Codonella*, *Tintinnus* and *Xystonellopsis* were present during all the three seasons. Other genera recorded were *Metacylis*, *Ascampbelliella*, *Parafavella*, *Coxiella*, *Dadayiella*, *Ormosella*, *Proplectella*, *Protorhabdonella*, *Rhabdonella*, *Epiplocyclis*, *Salpingella*, *Luminella*, *Stenosemella*, *Tintinnidium*, *Helicostomella*,

Leprotintinnus, *Daturella*, *Undella* and *Climacocylis*. Sarcodines were represented by radiolarians, acantharians and foraminiferans. Apart from these, flagellates consisted of *Peridinium*, *Ceratium*, *Dinophysis*, *Goniaulax*, *Noctiluca*, Silicoflagellates and *Prorocentrum*. Metazoa were mostly copepod nauplii, early copepodite stages, larval stages of appendicularians, polychaetes, chaetognaths and eggs of copepods and fishes.

Seasonal abundance of microzooplankton

Microzooplankton abundance during the three seasons in the central and eastern Arabian Sea is shown in Figures 2a,b. Average cell concentration of microzooplankton was highest during inter-monsoon (700 l^{-1}) followed by summer (300 l^{-1}) and winter (130 l^{-1}) seasons and average integrated column values were $143,000 \text{ m}^{-2}$, $66,000 \text{ m}^{-2}$ and $24,100 \text{ m}^{-2}$ respectively. Protozoa contributed 55–91% to the total microzooplankton. Among protozoa, flagellates (avg. 47%) were abundant followed by loricates (27%), aloricates (21%) and sarcodines (5%). Copepod nauplii and early copepodite stages of copepods formed the major component of the metazoan microzooplankton. Higher abundance of microzooplankton was found in the upper 100 m compared to 100–200 m depth except at stations J5 and J6 during inter-monsoon period. Below 100 m flagellates and aloricate ciliates were usually more common compared to other taxa. Microzooplankton density was higher in open ocean waters during inter-monsoon and winter while it was more at coastal stations during summer. During inter-monsoon there was not much variation in abundance between northern (avg. 700 l^{-1}) and southern (avg. 716 l^{-1}) stations, but during winter and summer seasons southern areas showed more abundance (351 l^{-1} and 180 l^{-1} respectively) than north (78 l^{-1} ; 140 l^{-1}).

Microzooplankton carbon ranged from 7 to $36 \mu\text{g C l}^{-1}$ (avg. $19 \mu\text{g C l}^{-1}$), 4 to $22 \mu\text{g C l}^{-1}$ (avg. $10 \mu\text{g C l}^{-1}$) and 4 to $25 \mu\text{g C l}^{-1}$ (avg. $12 \mu\text{g C l}^{-1}$) during inter-monsoon, winter and summer respectively. Southern stations showed comparatively higher biomass (avg. 3.96 g C m^{-2}) than the northern stations (1.96 g C m^{-2}). Microzooplankton biomass in terms of carbon was more than that of mesozooplankton in all seasons except in open ocean waters during winter monsoon when they were comparable (Table 1).

Discussion

Numerically, microzooplankton was dominated by protozoa which contributed on an average >75%. This is comparable to the results reported from Plymouth waters¹¹ where protozoa contributed to >97% of counts in

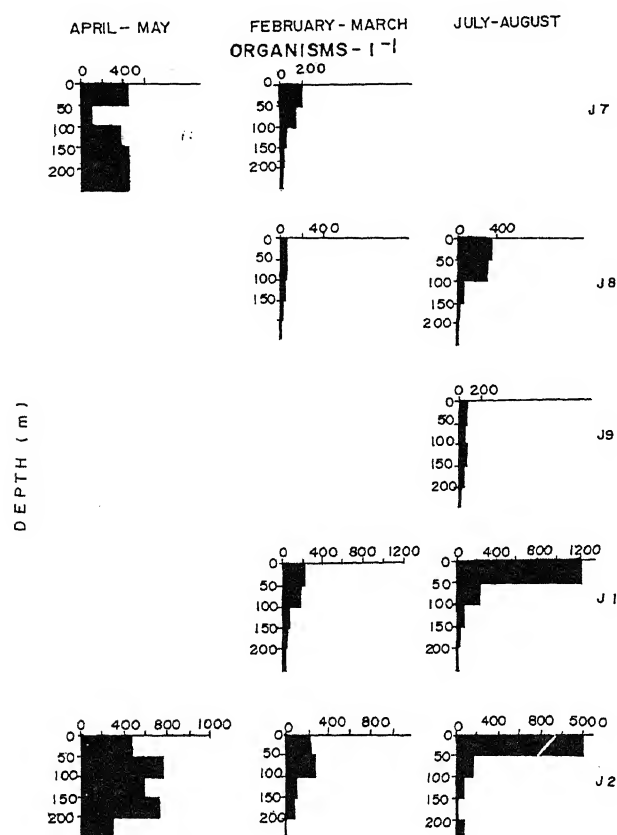


Figure 2a. Microzooplankton abundance during inter-monsoon, winter monsoon and summer monsoon at coastal stations (J1, J2, J7, J8 and J9).

the water column. Ciliates and flagellates dominated in the present study as reported¹¹.

An interesting finding of this study was the contribution of flagellates which ranged from 11 to 69% to the total microzooplankton. This suggests that they form an important group of microzooplankton in tropical waters similar to subtropical and temperate waters^{1,36-38}. Tintinnid population varied from 20 to 1060 l^{-1} in the present study, is less than that reported during the north Atlantic bloom (300–1600 l^{-1})² but higher than

Table 1. Average carbon content ($g C m^{-2}$) of microzooplankton and mesozooplankton

	Inter- monsoon	winter monsoon	Summer monsoon
Microzooplankton			
Open ocean stations	3.1	1.8	2.3
Coastal stations	5.48	2.1	2.4
Mesozooplankton			
Open ocean stations	1.51	1.89	0.87
Coastal stations	0.92	0.90	1.00

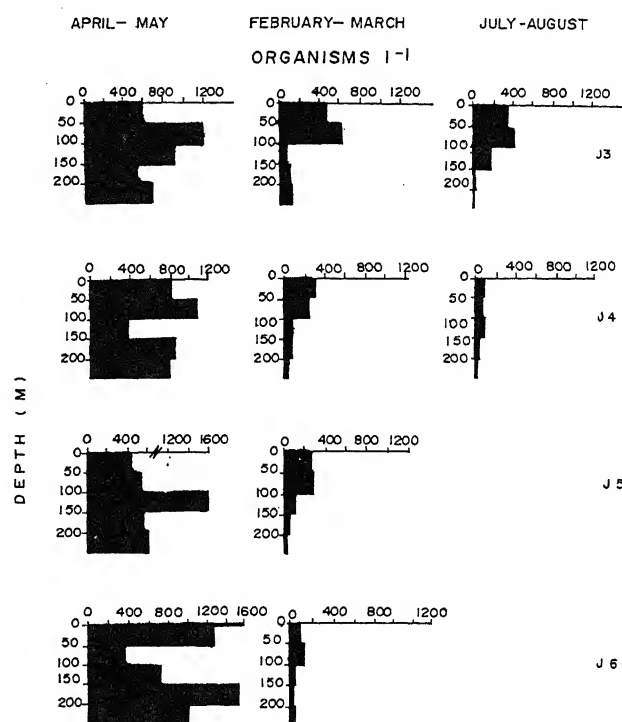


Figure 2b. Microzooplankton abundance during inter-monsoon, winter monsoon and summer monsoon at open ocean stations (J3-J6).

records from the Pichavaram Mangrove (4–13 l^{-1})²⁰, South India. Similarly, microzooplankton biomass of this study (4–36 $\mu g C l^{-1}$) is comparable to that of Lancaster Sound 10, where microzooplankton (>35 and <200 μm) biomass varied from 1.33 $\mu g C l^{-1}$ to 48.7 $\mu g C l^{-1}$. The assumption of taking cell volume of tintinnids as 50% of lorica volume might have led to some over-estimation³⁹.

The physical and chemical environments during the three cruises were different. During February, winter cooling led to increased mixed layer depths and availability of nutrients in euphotic zone enhancing primary production in the northern region⁴⁰. During April-May, the entire study area was oligotrophic whereas in August enhanced production was noticed in coastal waters as a result of upwelling. Interestingly, maximum population of microzooplankton occurred during inter-monsoon season when primary production and chlorophyll were low, but bacterial population was high compared to other seasons⁴¹. It would seem that the microzooplankton population increased through a microbial loop during this season by actively feeding on bacteria⁴². Higher microzooplankton abundance below 100 m at some stations during inter-monsoon coincided with trends in distribution of bacteria. The higher population of microzooplankton in coastal waters during summer monsoon might have resulted from the fresh water plumes⁴³ due to river run-off.

The fact that microzooplankton biomass in terms of carbon usually exceeded that of mesozooplankton is a pointer that they dominate the food chain in grazing and thereby phytoplankton losses as reported from the Atlantic^{1,2}. Future studies should concentrate on this since this implies that it forms the largest sink for primary production.

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Bacterial abundance and production in the central and eastern Arabian Sea

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Seasonal and spatial variations in bacterial and picoplankton abundances and bacterial production (thymidine incorporation rates) were determined in the water column up to 150 m in several stations in the central and eastern Arabian Sea. Higher bacterial densities of about 1×10^9 cells L^{-1} were observed during the intermonsoon periods of September and April/May compared to the southwest monsoon period of July/August and the winter period of February/March. Although primary production was low during April/May, bacterial production was much higher during this period unlike July/August. It also showed an increase from the northern to the southern Arabian Sea, suggesting the presence of high amounts of dissolved organic carbon in this area. High picoplankton densities, ranging up to 45×10^6 cells L^{-1} were observed during February, particularly in the northern Arabian Sea. Rapid turnover of bacteria during the intermonsoon period of April/May suggests the predominance of a 'microbial loop' in the foodweb and a prevailing source of dissolved organic carbon in the oceanic waters.

THE Arabian Sea is characterized by several unique seasonal and spatial variations in terms of biological, physical and chemical features which would directly influence microbial processes. Enormous amounts of particulate and dissolved organic carbon (POC and DOC) become available to bacteria during the southwest (summer) and northeast (winter) monsoons owing to enhanced primary production^{1,2}. Wind-driven upwelling during the southwest monsoon from June to September results in extensive areas of high primary production along the northwestern regions and the south and central west coast of India^{1,3-5}.

In addition, distinct differences between the northern and southern Arabian Sea will be reflected in the bacterial dynamics. High primary production is sustained in the northern Arabian Sea even after the southwest monsoon owing to cold and dry winds which result in convective mixing^{1,3,6}. While primary production decreases towards the south², DOC tends to increase from the north to the south. The subsurface waters of the northern Arabian Sea display a pronounced oxygen minimum layer associated with high nitrite maxima. The high-nitrite ridge extends from the continental margin off the north into the relatively oligotrophic central Arabian Sea. Primary production, *per se*, thus appears

to be decoupled from microbial processes that lead to denitrification⁷.

The months of March to May are periods of low primary production in the Arabian Sea³ and the possible effects of alternating eutrophy and oligotrophy on bacterial dynamics in the Arabian Sea, suggested by Azam *et al.*⁸ would be particularly felt during this season.

A few attempts have been made in recent years to study bacterial activities and picoplankton in the Arabian Sea⁹⁻¹². However, these studies have largely been confined to the monsoon period of July to October and the upwelling areas in the northwest Arabian Sea. Bacterial dynamics during the other seasons and parts of the Arabian Sea still remain poorly investigated.

The present study, as a part of the Joint Global Ocean Flux Studies-India (JGOFS) attempts to address: (1) Bacterial populations and production during the monsoon and intermonsoon periods, (2) Differences between the northern and southern Arabian Sea, and (3) Picoplankton abundance.

Materials and methods

Bacterial abundance

Four cruises were undertaken on board ORV *Sagar Kanya*: (1) Cruise SK #87 during September 1993; (2) SK #91 during April-May 1994 (3) SK #99 during February-March 1995, (4) SK #104 during 4 July-August 1995. Water samples were collected at various depths from different locations (Figure 1) using a CTD rosette sampler. Samples for microbiological studies were drawn from the rosette immediately after samples for dissolved gases were taken. These samples were handled, stored and analysed for bacterial abundances essentially according to the JGOFS protocols¹³ except for the addition of 0.22 μm filtered formaldehyde (3.7% final concentration) instead of 10% glutaraldehyde. Acridine orange direct counts (AODC) for bacteria were carried out following Parsons *et al.*¹⁴ and the JGOFS protocols. Up to 25 microscopic fields were counted for bacteria using oil immersion objectives (100 \times) under an Olympus BH2 epifluorescence microscope. Mean cell numbers per field were calculated and the number

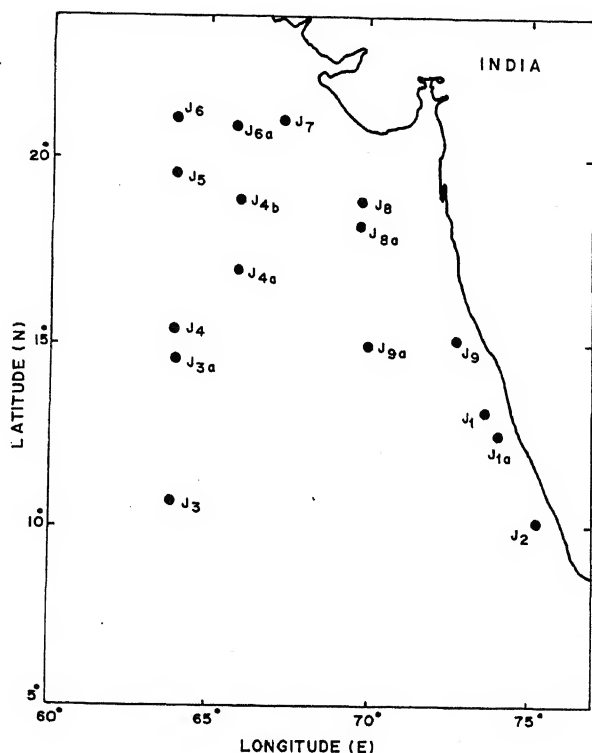


Figure 1. Location of sampling stations.

of bacterial cells L^{-1} determined based on the relationship detailed in Parsons *et al.*¹⁴.

3H (tritiated) thymidine incorporation rates

Incorporation of 3H -methyl (tritiated) thymidine (specific activity: 17,000 mCi/mmol; Bhabha Atomic Research Centre, Mumbai) by native bacteria was estimated by the method described in JGOFS protocol¹³ during cruises #SK 91 and #SK 104. Seawater samples from various depths, up to 150 m, were collected and analysed. A working solution of 21.25 ml containing 59 nmole of methyl 3H thymidine was prepared and 100 μ l of this solution was added to 27.8 ml of water sample to yield a final concentration of 10 nM thymidine. Samples were incubated for 1 h and the uptake was stopped by adding 37% formaldehyde to yield a final concentration of 3%. The aliquots were immediately filtered over 0.2 μ m Millipore filters and cold TCA precipitation and ethanol extraction carried out. Filters were immersed in 3 ml of liquid scintillation cocktail ('Cocktail-W', Spectrochem, Mumbai) and the samples assayed for radioactivity using a Packard 2500 TR liquid scintillation counter.

Bacterial production was estimated using mean oceanic conversion factor values of 2.17×10^{18} cells mol^{-1} thymidine incorporated and by converting the cell abun-

dance to carbon mass using a value of 2×10^{-14} g C per cell¹⁵.

Results

The observations presented in this paper pertain to four different seasons. The summer monsoon period in July/August 1995 was characterized by higher primary production and phytoplankton biomass, especially along the west coast of India (see Bhattathiri *et al.*, this issue). Decay of phytoplankton blooms occurred during September 1993 (SK #87). Primary production and phytoplankton biomass were also quite high in the northern latitudes during the winter month of February 1995 (SK #99). The intermonsoon period in April/May 1994 had the lowest levels of primary production and phytoplankton biomass of all the seasons sampled.

The highest bacterial densities, ranging from 0.34 to 1.5×10^9 cells L^{-1} were observed at the end of the southwest monsoon (September 1993) at the three stations sampled in the northern Arabian sea along $66^\circ E$ (Figure 2). Subsurface maxima were evident in all these locations. High bacterial densities were also observed

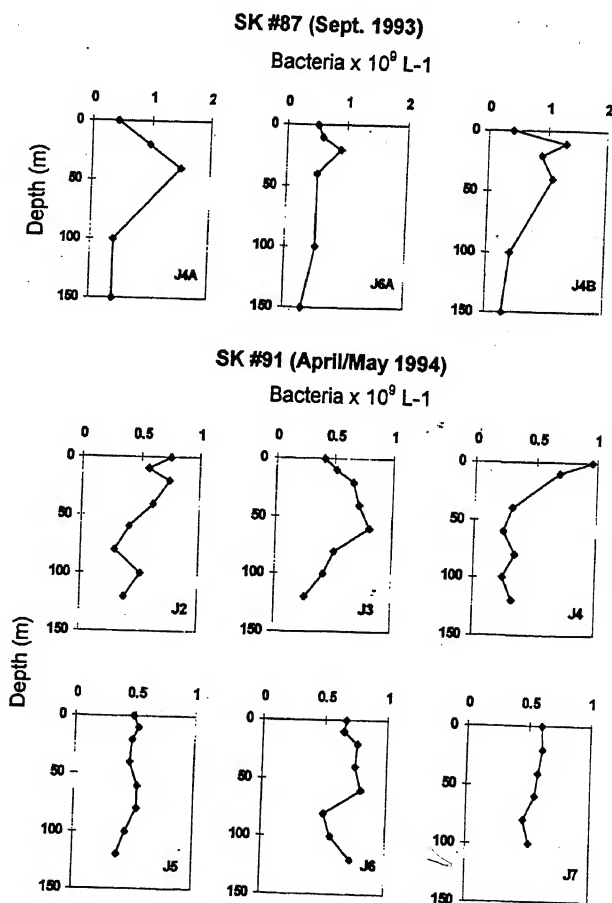


Figure 2. Bacterial abundance during cruises SK #87 (September 1993) and SK #91 (April/May 1994).

in the intermonsoon period of April–May 1994 (Figure 2; range: $0.24\text{--}0.96 \times 10^9 \text{ L}^{-1}$). However, no sharp sub-surface maxima were detected at any of the locations during this period. The coastal stations (for example, J2 and J7) and the offshore stations in the central Arabian Sea (J3 and J4) had similar bacterial abundances. Bacterial numbers were more or less similar from north to south along 64°E (Figure 2). Bacterial densities during the summer monsoon (SK #104) were fairly high, ranging from 0.13 to $0.64 \times 10^9 \text{ L}^{-1}$ (Figure 3). As in the intermonsoon season, bacterial abundances in coastal stations (J2, J8A and J9) and in the open oceanic stations (J3 and J3A) were similar. These numbers, however, were slightly lower than those observed in September and April. In contrast, bacterial abundance was about an order of magnitude lower (0.05 to $0.09 \times 10^9 \text{ L}^{-1}$) during February 1995 in all sampling stations, except the northernmost, coastal station J8 (Figure 4).

Thymidine incorporation rates were measured during the intermonsoon (SK #91) and the summer monsoon (SK #104) cruises (Figures 5 and 6). During the former, higher uptake rates, up to 25 to $50 \text{ pmol L}^{-1} \text{ h}^{-1}$, were observed in the southern coastal station J1 and the southern offshore stations, J3 and J4, all below the latitude of 15°N . Many of the stations showed higher rates at about $40\text{--}60 \text{ m}$ depths. Uptake rates during summer monsoon ranged from 0.2 to $3 \text{ pmol L}^{-1} \text{ h}^{-1}$, about an order of magnitude lower than those observed in the intermonsoon (Figure 6). The highest uptake rate during this season occurred in the southern coastal station J1A. Bacterial production increased with depth in all the stations except J1A.

Bacterial production (BP) exceeded primary production (PP) throughout the 120 m water column in 3 out of the 4 stations during intermonsoon (Figure 7). PP in the

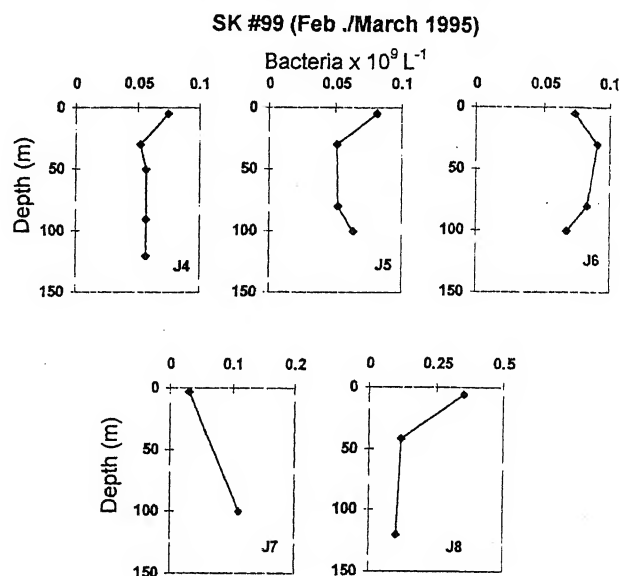


Figure 4. Bacterial abundance during cruise SK #99 in February/March 1995.

upper 50 m at some of these stations was only about 2% of the BP. In contrast, PP during the summer monsoon far exceeded the BP till a depth of 40 m (Figure 7), BP contributing only ca. 10% of the PP.

High picoplankton abundance, with a maximum of up to $60 \times 10^6 \text{ cells L}^{-1}$ was observed in the northern stations during February/March 1995, compared to ca. $10 \times 10^6 \text{ cells L}^{-1}$ in the more southern, oceanic stations (Figure 8). Picoplankton densities were similar to these oceanic waters during summer monsoon. However, the southernmost coastal station J2 harboured high numbers of picoplankton similar to coastal stations during February/March 1995 (Figure 8).

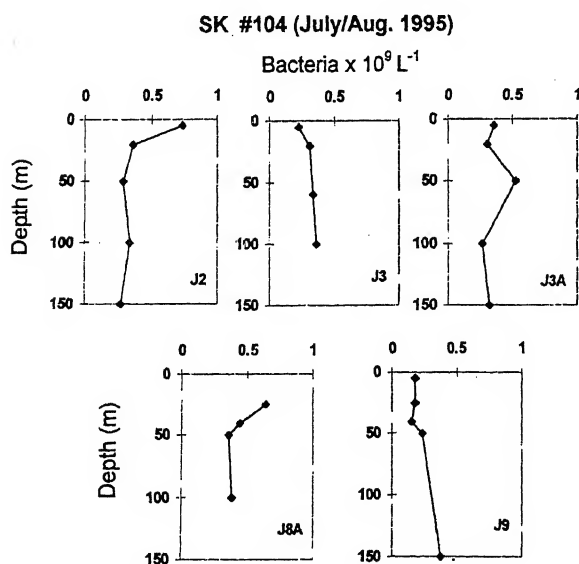


Figure 3. Bacterial abundance during cruise SK #104 in July/August 1995.

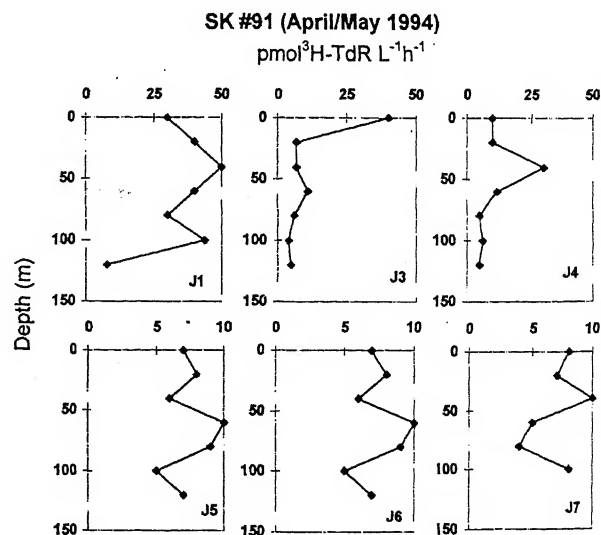


Figure 5. Thymidine incorporation rates during cruise SK #91 in April/May 1994.

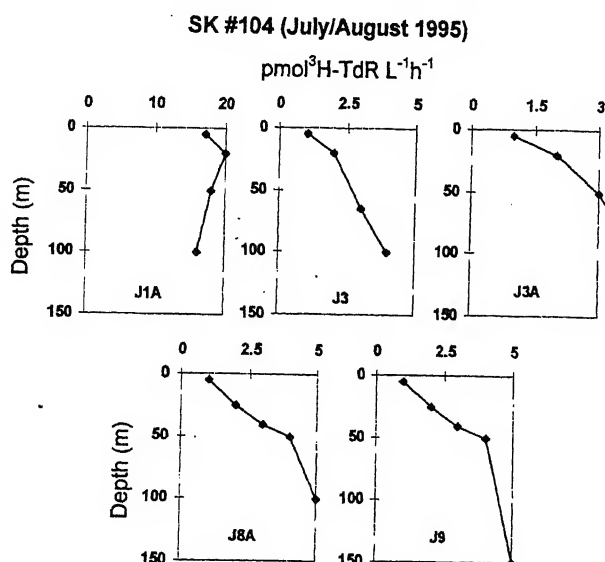


Figure 6. Thymidine incorporation rates during cruise SK #104 in July/August 1995.

Discussion

Peak bacterial abundances were not related to seasons of high primary productivity during the summer or winter monsoons. Thus, the highest bacterial densities were observed at the end of the southwest monsoon in September (Figure 2). Although only three stations at the northern Arabian Sea were sampled during this period,

the high bacterial densities observed during this period are probably typical of this season. The values measured in this study are similar to those reported by Ducklow⁹ ($>1 \times 10^9 \text{ L}^{-1}$) for the Northern Arabian Sea and the Gulf of Oman during September 1994. Likewise, although a pronounced enhancement of primary production and phytoplankton biomass and high picoplankton abundance with a distinct north to south gradient occurred along 64°E as a result of winter cooling and convective mixing⁵ (Figure 8), bacterial densities were not related to such high primary production and were the lowest during this season for the Arabian Sea.

Bacterial abundance increased substantially by April/May at all stations sampled (Figure 2). This period is generally known to be a season of low primary productivity and oligotrophic conditions in the Arabian Sea^{3,16}. Increase in bacterial abundance during the two intermonsoon periods in September and April/May might be related to a decay of the earlier phytoplankton blooms, increasing phytoplankton exudation and particle breakdown at the end of the season⁹. Our observations during April/May suggest that bacteria during this season responded to the phytoplankton blooms of February/March only after a pronounced lag period¹⁵. In the equatorial Pacific, Kirchman *et al.*¹⁷ observed a lack of covariance and an uncoupling between primary and bacterial productions over a similar time scale of about 1–2 months. Azam *et al.*⁸ suggested that following a phytoplankton bloom, labile DOC would be rapidly utilized by bacteria, while the slow-to-degrade DOC would

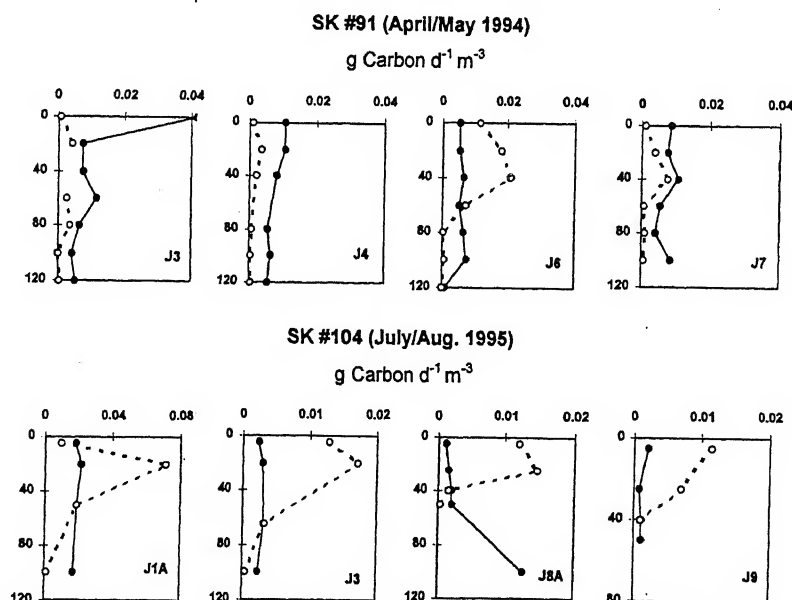


Figure 7. Bacterial (continuous lines) and primary (stippled lines) production during cruises SK #91 in April/May 1994 and SK #104 in July/August 1995.

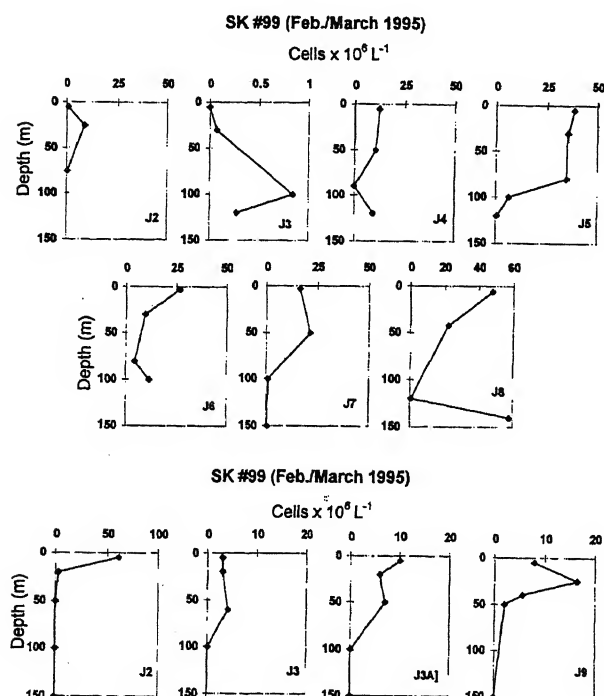


Figure 8. Picoplankton abundance during cruises SK #99 in February 1995 and SK #104 in July/August 1995.

sustain bacteria during oligotrophic conditions.

Bacterial production also was much higher during April/May than during July/August. Although bacterial production is normally about 20% of the primary production, values up to 80% of the primary production have been recorded in equatorial Pacific¹⁷. Our observations indicate that biological production during the premonsoon period of April/May was dominated by bacteria, phytoplankton production often accounting for only about 2% of the BP (Figure 7). This is in contrast to the summer monsoon period, when bacterial production was lower and comprised only about 18% of the phytoplankton production. It is not yet clear from our studies as to why bacterial production increased towards the south during April/May. Chandralata Raghukumar (pers. commun.) observed patches of *Trichodesmium* (red tide) blooms in some stations in this area. It is likely that a vigorous red tide bloom preceding our observations provided DOC to promote bacterial dynamics during this season. Such *Trichodesmium* blooms are common in March–April in the Arabian Sea¹⁸.

Our observations might help to provide an explanation to the zooplankton paradox of the Arabian Sea¹⁹. Zooplankton biomass remains more or less constant throughout the year in the Arabian Sea despite variations

in primary productivity leading to the suggestion that secondary production in the Arabian Sea might be sustained through the 'microbial loop'²⁰ during 'lean' seasons. The rapid bacterial turnover in April/May is an evidence that flagellate and ciliate grazing during this period might be intense and further sustain the zooplankton²¹.

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Photosynthetically available radiation in the central and eastern Arabian Sea

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In this article, we present the analysis of the photosynthetically available radiation (PAR, 400–700 nm) in the eastern and central Arabian Sea (21°30' N, 64°E to 12°N, 74°E) during February–March, 1995 using data collected with quantum PAR sensor and maritime solar radiation models for clear sky. The peak values observed were in the range of about 1700 to 1830 $\mu\text{mole s}^{-1}\text{m}^{-2}$ or equivalent to about 365 to 435 Wm^{-2} . The radiative transfer models of Gregg and Carder, and Frouin *et al.* were used to investigate the relationship between the quantum and energy of solar radiation available in the PAR region. The average ratio of this quanta per energy was found to be about 4.2 $\mu\text{mole W}^{-1}\text{s}^{-1}$. Aerosol optical depth obtained using the sunphotometer at 498 nm for the region was found to vary from 0.07 to 0.19.

LIFE in the ocean is dependent on the amount of sunlight available. The amount of solar irradiance reaching the ocean surface is important in all disciplines of oceanography. From a physical oceanography point of view, the total incident solar irradiance constitutes a major boundary forcing for ocean circulation and determines meridional heat transport. From the biological point of view, solar irradiance in the photosynthetically active interval PAR, regulates marine primary productivity and therefore the evolution of aquatic ecosystems^{1,2}.

The Arabian Sea is a highly productive oceanic region. One of the goals of JGOFS is to understand the process in controlling the carbon flux in the Arabian Sea. Its unique feature is the regular oscillation of high rates of primary production under relatively constant levels of solar radiation³. Though the sunlight is available on the surface at a wide spectral range, the useful light available in the upper layers of ocean is limited in PAR region in the range of 350–700 nm. PAR is defined in terms of energy (Wm^{-2}) as

$$E_{\text{PAR}}(z) = \int_{350}^{700} Ed(\lambda, z) d\lambda, \quad (1)$$

where $Ed(\lambda, z)$ is the downward spectral irradiance (Wm^{-2}) at wavelength λ (nanometers) at depth z (meters). The total quanta available in the photosynthetic

range is given by

$$Q_{\text{PAR}}(z) = 1/hc \int_{300}^{700} \lambda Ed(\lambda, z) d\lambda, \quad (2)$$

where h is the Planck's constant and c is the velocity of light in vacuum. Q_{PAR} has units of quanta $\text{s}^{-1}\text{m}^{-2}$.

Usually PAR is taken as a constant fraction of total solar irradiance. PAR includes more than 45% of total radiation reaching the earth's surface for low zenith angle or high elevation above 30°.

Materials and methods

During February–March, 1995 PAR values were measured in the eastern and central Arabian Sea (21°30' N, 64°E to 74°E) under the JGOFS (India) programme aboard the research vessel ORV Sagar Kanya (Table 1). Q_{PAR} values were measured using quantum PAR sensor LI-190S (LI-COR Inc., USA) (Figure 1). The sensor was placed at a clear site on a raised platform on the ship to obtain sunlight without any obstruction. The PAR range of this sensor is 400–700 nm. The unit is given in $\mu\text{mole s}^{-1}\text{m}^{-2}$. (1 $\mu\text{mole s}^{-1}\text{m}^{-2} = 6.022 \cdot 10^{17}$ quanta $\text{s}^{-1}\text{m}^{-2}$). E_{PAR} is evaluated using two solar radia-

Table 1. Stations covered in Arabian Sea for the measurements of PAR data

Station	Data	Latitude N	Longitude E
1	12.2.95	21° 22'	64° 10'
2	13.2.95	21° 25'	64° 16'
3	21.2.95	15° 00'	65° 50'
4	22.2.95	14° 58'	63° 49'
5	23.2.95	14° 55'	63° 48'
6	25.2.95	11° 00'	64° 00'
7	26.2.95	10° 55'	65° 30'
8	1.3.95	11° 40'	74° 20'

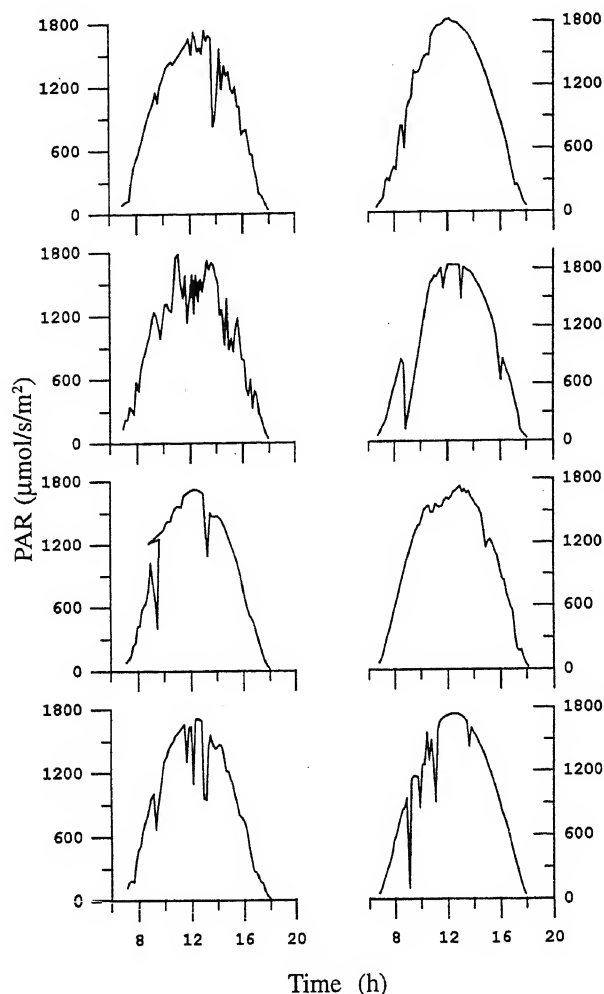


Figure 1. PAR data using sensor LI-190 at different stations.

tion models of Gregg and Carder⁴, and Frouin *et al.*⁵. The meteorological parameters were measured with an automatic logging meteorological station^{6,7}. The atmospheric pressure recorded every minute was averaged over a ten minutes interval, while wind speed, wet and dry bulb temperatures were recorded every 3 hours. A single wavelength hand held sunphotometer was used to obtain the aerosol optical depth (wavelength 498 nm, FWHM = 13 nm). The unit was calibrated using the Langley method at a clear sight at a high altitude station, Mount Gurushikhar.

Since all measurements were made with reference to Indian Standard Time (Longitude 82° 30' E), corrections were applied to obtain the local time at the meridian of observation. In the model, airmass is assumed to be unity, which is typical for maritime atmospheres. Ozone and water vapour contents were not measured. Average values for water vapour were obtained from the atlas of Ramesh Kumar *et al.*⁸, average ozone content was taken as 320 Dobson Units, which gives ozone scale height to

be 0.32 cm. Total precipitable water vapour was taken from the atlas⁹ as 5.5 g cm⁻².

Solar radiation models

Spectral model of Gregg and Carder¹

The high resolution solar spectral irradiance model of Gregg and Carder⁴ was used to evaluate E_{PAR} . This model was adopted as it was suited for maritime atmosphere and it provided spectral information at a high resolution of 1 nm. The model computed contributions from direct and diffuse sunlight available at any time in the range of 350–700 nm. We have used the range of 400–700 nm for compatibility with the quantum sensor output.

Attenuation of solar irradiance in the visible and near-UV wavelengths is attributed to atmospheric processes such as scattering by gas mixture (Rayleigh scattering), absorption by gas mixture, absorption by ozone, and scattering and absorption by water vapour. Irradiance that is not scattered out of the direct beam but toward the surface is the diffuse irradiance. The sum of the direct and diffuse components defines the global irradiance available for use.

$$E_{dd}(\lambda) = F_0(\lambda) \cos(\theta) Tr(\lambda)$$

$$Ta(\lambda) Toz(\lambda) To(\lambda) Tw(\lambda), \quad (3)$$

$$E_{ds}(\lambda) = I_r(\lambda) + I_a(\lambda), \quad (4)$$

where dd and ds refer to direct and diffuse components, $F_0(l)$ is the mean extra terrestrial irradiance corrected for earth-sun distances and orbital eccentricity, θ is the solar zenith angle, Tr , Ta , Toz , To and Tw represent transmittance after absorption or scattering by air molecules, aerosol, ozone, oxygen and water vapour respectively. Similarly I_r and I_a represent the diffuse components due to Rayleigh scattering and aerosol.

For maritime environment, the above equations on incorporating air-sea interactions change to

$$E_{dd}(\lambda) = E_{dd}(\lambda) \cdot (1 - \rho_d), \quad (5)$$

$$E_{ds}(\lambda) = E_{ds}(\lambda) \cdot (1 - \rho_s), \quad (6)$$

where ρ_d is the direct sea surface reflectance and ρ_s is the diffuse reflectance.

Aerosol optical depth

Marine aerosols, being larger than continental aerosols, reduce solar radiation by absorption and large scattering. Total optical depth is a sum of optical depths due to Rayleigh scattering, (τ_R) aerosol extinction (τ_A) and gaseous absorption (τ_G). Total columnar optical depth

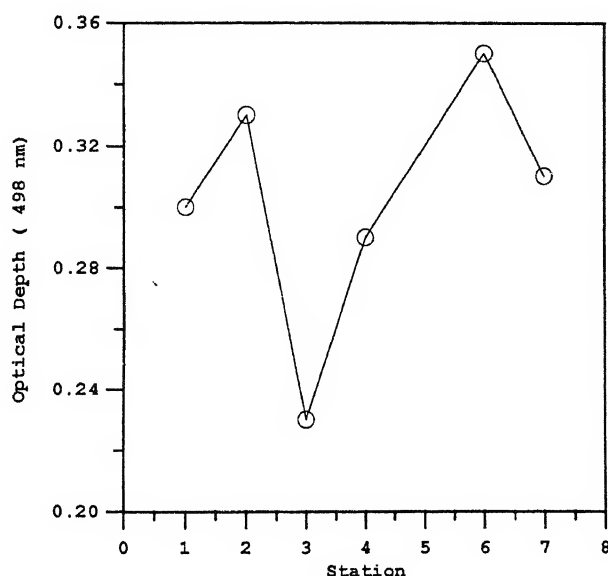


Figure 2. Total columnar optical depth at various stations

measured at 498 nm using the sunphotometer (Figure 2) is given as

$$\tau(\lambda) = \tau_R(\lambda) + \tau_A(\lambda) + \tau_G(\lambda). \quad (6)$$

Rayleigh optical depth is expressed as^{10, 11}

$$\tau_R(\lambda) = (p/p_0) 0.00865 \lambda^{-(3.916+0.074\lambda+0.050/\lambda)}. \quad (7)$$

Here λ is given in micrometer, p is the barometric pressure (mb) and p_0 is the standard atmospheric pressure ($p_0 = 1013.25$ mb).

Optical depth component due to ozone is calculated at $\lambda = 498$ nm and is given by $\tau_{O_3}(498) = 0.01$ and contributions due to other gases are assumed to be the same as at 484 nm, whose value is given as, $\tau_G(484) = 0.005$. Using the equation (7) for τ_R at 498 nm and optical depth for gases as given above, we calculate the aerosol optical depth $\tau_A(498)$ at each station. Since no data for optical depth was available at stations 8, for this station we have taken the average value of the total columnar optical depth of 0.3. With the help of aerosol optical depth at 498 nm, we determine the aerosol depths at other wavelengths using the Angstrom formula

$$\tau_A(\lambda) = \beta \lambda^{-\alpha} \quad (\lambda \text{ in } \mu\text{m}). \quad (8)$$

Here β is the turbidity coefficient representing the aerosol concentration and α is the Angstrom component.

Angstrom component α is given in terms of Junge exponent γ as¹³

$$\alpha = -(\gamma + 3). \quad (9)$$

Aerosol size distributions can be expressed by two expressions, Junge distribution¹⁴ and by an expression dependent on relative humidity and wind speed¹⁵. The latter expression is evaluated for three radii of particles, 0.1, 1.0 and 10 nm. Logarithmic regression of these with the three radii is computed and the regression coefficients give the Junge exponent. Using equation (9) we then obtain the Angstrom component. Having obtained α , β can be obtained from equation (8) with the observed value of $\tau_A(498)$. $\tau_A(\lambda)$ is then calculated for all other wavelengths.

Model of Frouin *et al.*

This is a simple, yet accurate analytical formula to determine the PAR at the ocean surface under clear skies⁵. The formula is given as follows

$$E_{PAR} = F_{PAR} T_1 T_2 T_3 \quad (10)$$

F_{PAR} is the monochromatic extraterrestrial irradiance integrated over 400–700 nm, taking into consideration the earth–sun separation.

$$T_1 = \frac{\cos(\theta) \exp[-a+b/V]/\cos(\theta)}{1-\rho(a'+b'/V)}, \quad (11)$$

$$T_2 = \exp[-av(U_v/\cos(\theta))^{bv}], \quad (12)$$

$$T_3 = \exp[-a_0(U_o/\cos(\theta))^{b_0}], \quad (13)$$

ρ is average surface reflectance, V visibility, U the vertically integrated absorbed amount, θ the zenith angle, and subscripts o and v denote ozone and water vapour respectively.

The regression coefficients for maritime atmosphere over spectral region 400–700 nm are given as

$$a = 0.068 \quad b = 0.379 \quad a' = 0.117, \quad b' = 0.493,$$

$$av = 0.002, \quad bv = 0.87 \quad a_0 = 0.052 \quad b_0 = 0.99.$$

Visibility V (km) is calculated using the aerosol optical depth data. Aerosol extinction coefficient at 550 nm is expressed in terms of visibility¹⁶

$$C_a(550) = 3.91/V. \quad (14)$$

This extinction coefficient is related to aerosol optical thickness by

$$\tau_A(550) = C_a(550) H_a, \quad (15)$$

where H_a is the aerosol scale height, which is assumed to be 1 km (ref. 17). We can determine aerosol optical depth at 550 nm using the method described earlier in Gregg and Carder⁴ model.

Results and discussion

The two models were evaluated for eight stations in the Arabian Sea. They were found to agree closely with the real time data collected using a quantum PAR sensor. With the exception of ozone and water vapour contents, all other meteorological parameters required for the models were measured at the stations. The measured peak values of PAR were found to vary from about 1700 to 1830 $\mu\text{mol s}^{-1} \text{m}^{-2}$ or equivalent to about 365 to 435 W m^{-2} evaluated using the solar radiation models. Figure 3 shows the measured and model data given for a station. In the absence of observed meteorological data for the duration of calculation, the last observed values were considered, assuming that variations over the period were not significantly large. The models seem to agree better at stations when there were less disturbances in PAR values, such as at station 11°N, 64°E

(25 February) and 11° 40'N, 74° 20'E (3 March). A discontinuity was usually observed with the quantum sensor data around 1000 hours, which is attributed to the shadow of a mast on the deck while the others at 1300 to 1500 hours probably caused by clouds. Since the models were tuned for clear sky, the observed values were filtered to remove all such spurious data.

We have used the models to analyse the behaviour of $Q_{\text{PAR}} : E_{\text{PAR}}$. The ratio of observed PAR quantum values to the solar irradiance values evaluated using the models of Gregg and Carder⁴ and Frouin *et al.*⁵ were 4.08 and 4.04 respectively (Figures 4 and 5). After filtering the data for disturbances, the ratios were boosted to values 4.24 and 4.20 for the Gregg and Carder and Frouin *et al.* models respectively. The difference in the ratios for both the models was found to be marginal ($< 0.02\%$), when the solar irradiance was estimated for zenith angles below 68°, 80° and 90°. Morel and Smith¹⁸ obtained a value of 2.77×10^{18} quanta $\text{W}^{-1} \text{s}^{-1}$ or 4.6 $\mu\text{mol W}^{-2} \text{s}^{-1}$ for 400–700 nm. ($1 \mu\text{mol s}^{-1} \text{m}^{-2} = 6.022 \cdot 10^{17}$ quanta $\text{s}^{-1} \text{m}^{-2}$). Their observations were for solar zenith angles $< 68^\circ$ and the ratio is for monochromatic radiant energy at 550 nm, the central wavelength of the spectral region 400–700 nm. Baker and Frouin¹⁹ obtained a ratio of 2.68×10^{18} quanta $\text{W}^{-1} \text{s}^{-1}$ or 4.38 $\mu\text{mol W}^{-1} \text{s}^{-1} \text{d} \lambda$ is taken as the central wavelength, in the range 350–700 nm.

Investigation of aerosol content is also part of the study under JGOFS (India) and it is also an important parameter required for remote sensing, validation of algorithms and models. Atmospheric turbidity obtained using sunphotometer at 498 nm was found to vary from

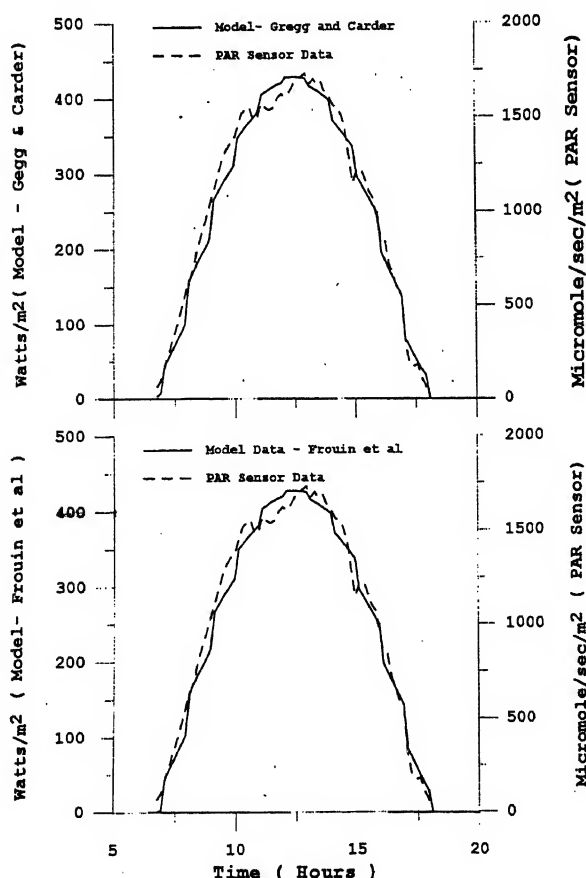


Figure 3. PAR sensor and model data for station 6 (25 March).

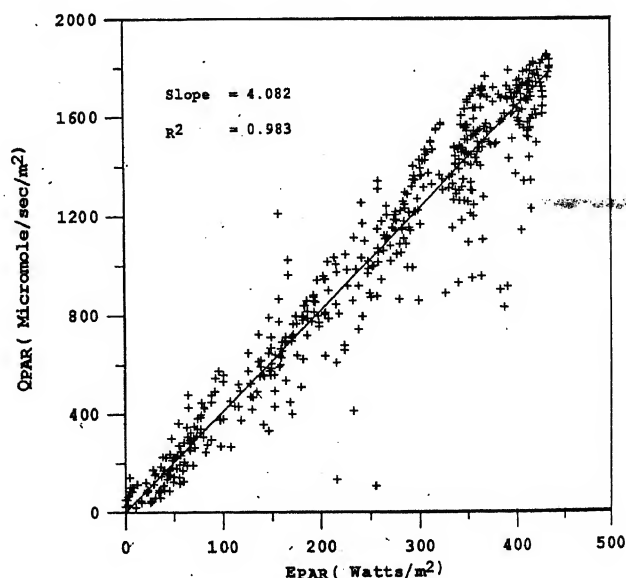


Figure 4. PAR sensor data vs solar irradiance model of Gregg and Carder.

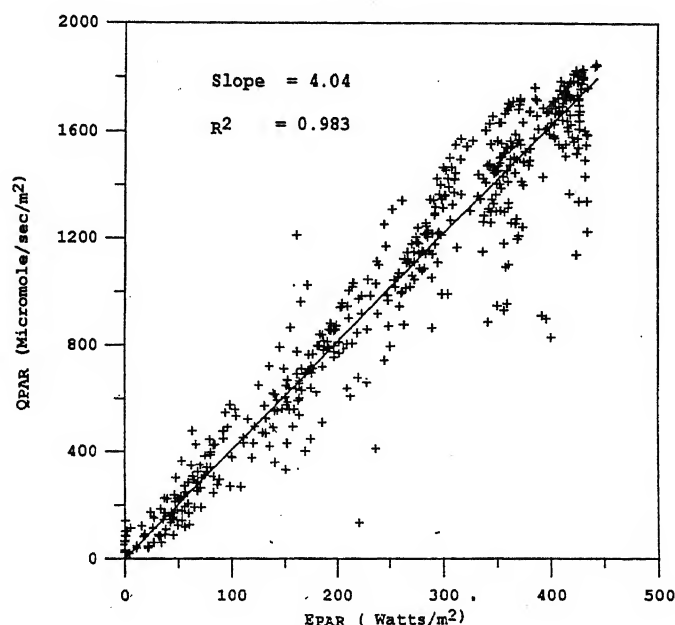


Figure 5. PAR sensor data vs solar irradiance model of Frouin *et al.*

0.23 to 0.35. The aerosol optical depth derived from this was found to vary from 0.07 to 0.19.

The analytical model of Frouin *et al.*⁵ was found to be simple compared to the rigorous high resolution spectral model of Carder and Gregg⁴. The model of Frouin *et al.* could be used to obtain the much required parameter of aerosol optical depth. Solar irradiance values in PAR range can be measured using the PAR sensor and the same can be equated to the model of Frouin *et al.*² to obtain the aerosol optical depth.

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^{234}Th scavenging and particle export fluxes from the upper 100 m of the Arabian Sea

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We have determined the particle scavenging rates, export fluxes of ^{234}Th and settling particles from the upper 100 m of the Arabian Sea as a part of the JGOFS (India) Programme. The spatial and temporal measurements made in the open ocean profiles reveal close similarities in the dissolved $^{234}\text{Th} : ^{238}\text{U}$ disequilibria, suggesting that the rates of particle-associated scavenging processes are generally uniform in the central Arabian Sea. The observed disequilibrium integrated for the upper 100 m yields a mean scavenging residence time of ~ 30 days and a removal rate of $\sim 3400 \text{ dpm m}^{-2} \text{ d}^{-1}$ for ^{234}Th , from dissolved to particulate phases.

The deficiency of total ^{234}Th (dissolved + particulate) relative to ^{238}U allows us to compute the vertical export flux of particulate ^{234}Th . The flux data for the upper 100 m show spatial variations with enhanced export fluxes centered around 22°N 67°E , a region characterized by higher rates of column primary productivity. Using the ^{234}Th export fluxes and the measured specific activity of ^{234}Th in the sediment traps, we have computed the particle and carbon fluxes at 100 m. These results reveal that the particle fluxes determined from sediment traps are systematically low and the estimated C export fluxes are grossly out of proportion with the column primary production.

THE reactive nuclides of the U–Th decay series, viz. ^{234}Th , ^{210}Po and ^{210}Pb serve as valuable tracers for studying the rates of particle-associated chemical scavenging processes, particle export from the euphotic zone and new production in the ocean. These tracers are introduced in the water column as dissolved species (by radioactive decay of their parent nuclides dissolved in seawater) and are redistributed between the dissolved and particulate phases depending on their particle reactivity and availability of particle surfaces. The latter depends on the rates of primary production, particle transformation and their net downward export. Bhat *et al.*¹ were the first to demonstrate the application of ^{234}Th – ^{238}U disequilibrium to study the particle scavenging processes in oceanic surface waters. These studies were later expanded by Coale and Bruland^{2,3} and suggested that the scavenging rates of reactive elements may vary as a function of new production rather than total primary production. Recently, Buesseler *et al.*^{4,5} have used the profiles of ^{234}Th and a non-steady state scavenging model

to quantify the particulate ^{234}Th fluxes and to derive export fluxes of particulate carbon and nitrogen from the euphotic zone. Likewise, the ^{210}Po – ^{210}Pb disequilibrium in surface waters and the particulate release of ^{210}Po at ~ 100 m have been modelled to derive the new production rates in the Arabian Sea⁶.

Measurements of particle export fluxes using sediment traps in the top few hundred meters of the water column have also been compared with those derived based on the $^{234}\text{Th} : ^{238}\text{U}$ disequilibrium studies^{7–9}. These studies have shown significant discrepancy for the trap fluxes suggesting that the shallow traps do not always provide a reliable measure of the particle export. We report here our results on the ^{234}Th scavenging and particle export fluxes in the eastern-central Arabian Sea during the JGOFS (India) cruises. We have used the ^{234}Th and ^{238}U isotope data from unfiltered (total) samples and the specific activity of ^{234}Th in the sediment trap material to derive the particle export fluxes.

Samples and analyses

Samples were collected during ORV *Sagar Kanya* cruises No. 91 (April–May 1994), No. 99 (February–March 1995) and No. 104 (July–August 1995) in the Arabian sea (Figure 1). Three stations sampled during April–May 1994 at 11°N , 15°N and 22°N along the 64°E transect were reoccupied during the subsequent cruises to study the temporal and spatial variations in the export fluxes of ^{234}Th and settling particles. The hydrographic data (potential temperature, salinity and depth) were simultaneously measured at all stations. Parallel measurements of primary productivity were also made using *in-situ* incubations¹⁰.

Unfiltered seawater samples from the upper 300 m water column were collected using 30 litre Go-Flo bottles fitted on the CTD rosette and were analysed for ^{234}Th , ^{238}U , ^{210}Po and ^{210}Pb . At selected stations, along the 64°E transect (Figure 1), samples were also filtered through $0.4 \mu\text{m}$ pore size Nuclepore filters (47 mm diameter) directly attached to the Go-Flo bottles. All these nuclides were pre-concentrated from about 20–25 litres of either filtered or unfiltered seawater with $\text{Fe}(\text{OH})_3$ in presence of chemical yield tracers. Subsequently, radiochemical separation and purification of these

nuclides were achieved following the procedures of Sarin *et al.*¹¹.

The mass flux of settling particles at 100 and 300 m depths was measured using free-floating sediment traps deployed, during April–May 1994 (cruise-91) and February–March 1995 (cruise-99), for a period of 3–5 days at station sites 15°N, 64°E and 21.5°N, 64°E. The multi-traps (Hydrobios, Germany) consisting of a PVC cylinder (having collection area 0.01515 m² and aspect ratio of 1:4) fitted to six polyethylene bottles were used in this study. After recovery of the traps, supernatant water in the sample bottles was carefully decanted and 'swimmers' were removed. Samples were then filtered through 0.4 µm Nuclepore filters and dried at 40°C. One-half of the collected material was analysed for ²³⁴Th, ²¹⁰Po and ²¹⁰Pb. For this, filters were acid digested (HCl + HNO₃) in presence of yield spikes and analytical procedures follow that of Sarin *et al.*¹¹. The remaining half of the filters were stored for the analysis of organic carbon.

Results and discussion

In this article we present the data on the dissolved and total (dissolved + particulate) ²³⁴Th concentrations; results on the ²¹⁰Po and ²¹⁰Pb will be presented elsewhere. The distribution of dissolved and total ²³⁴Th activities vs depth at the three stations along the 64°E transect is shown in Figure 2. The vertical profiles of total ²³⁴Th measured at stations along the coast are presented in Figure 3. The errors ($\pm 1\sigma$) in the ²³⁴Th activities are

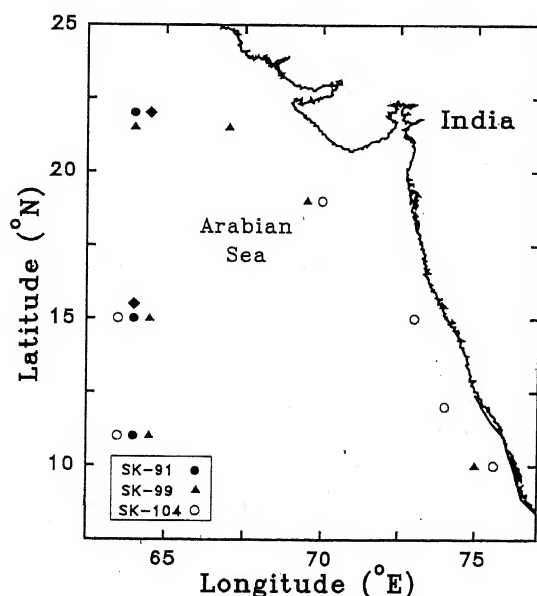


Figure 1. Stations sampled during three cruises: SK-91 (April–May 1994), SK-99 (February–March 1995) and SK-104 (July–August 1995). Reoccupation of the stations during different seasons, along the 64°E transect, is shown as an offset. Locations of trap deployment is shown by diamond symbol.

about $\pm 5\%$ arising from counting statistics, tracer calibrations and blank corrections. The accuracy and precision of our ²³⁴Th measurements were checked by analysing unfiltered seawater samples collected from 1000 to 2000 m depths. The ²³⁴Th/²³⁸U activity ratios measured in these samples were in close agreement to the expected equilibrium value. The ²³⁸U activity (Figures 2 and 3) was calculated based on its relation with salinity [²³⁸U (dpm/kg) = (0.06969) × S‰] derived for the Arabian Sea¹¹. This relation is based on measured ²³⁸U and salinity data for samples collected from surface to 2000 m depth including those from sub-oxic regions. The ²³⁸U concentration did not show any distinct decrease in the samples from denitrification region^{6,11}.

²³⁴Th scavenging

The dissolved activity of ²³⁴Th, at all sampling depths

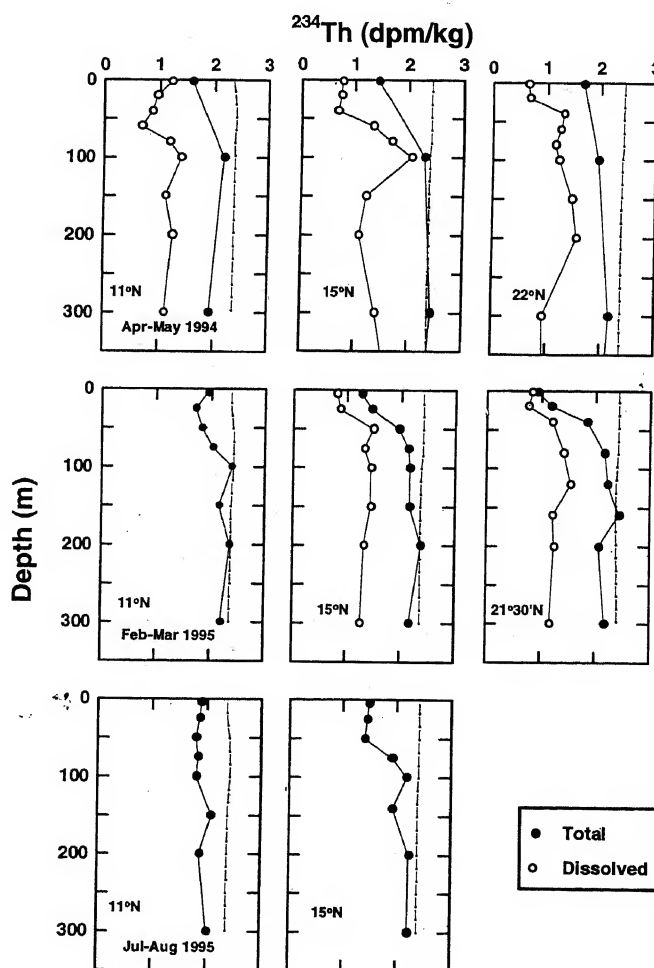


Figure 2. Vertical profiles of dissolved and total (dissolved + particulate) ²³⁴Th activities in the upper 300 m at three open ocean stations along the 64°E transect. The dash line represents the dissolved activity of the parent isotope ²³⁸U. A gross deficiency of ²³⁴Th is clearly evident in the surface 100 m.

(Figure 2), shows a pronounced deficiency with respect to its parent ^{238}U whereas total ^{234}Th shows near-equilibrium concentration at $>100\text{ m}$. The deficiency of dissolved ^{234}Th is attributed to its removal from the dissolved to particulate phases. The dissolved $^{234}\text{Th} : ^{238}\text{U}$ activity ratio in surface waters at three stations along the 64°E , during April–May (summer), ranges from 0.28 to 0.43; with lowest value occurring at the northern-most station. However, the column deficiency of dissolved ^{234}Th integrated over 0–100 m is quite similar at all the three stations. These results yield a mean residence time of about 30 days for dissolved ^{234}Th in the surface 100 m with respect to its removal onto particles. The nearly identical extent of dissolved $^{234}\text{Th} : ^{238}\text{U}$ disequilibrium suggests that the rates of particle-associated scavenging processes in the central Arabian Sea are quite uniform and are not resolvable based on ^{234}Th distribution.

These three stations along the 64°E transect were reoccupied during winter (February–March, Figure 1). The overall pattern of dissolved ^{234}Th distribution and the extent of $^{234}\text{Th} : ^{238}\text{U}$ disequilibrium during the winter

months is nearly the same as that measured during the summer. The mean dissolved $^{234}\text{Th} : ^{238}\text{U}$ activity ratio in the surface 100 m at these three stations is 0.49 (Figure 2), which yields ^{234}Th scavenging residence time of 34 days, quite similar to that observed during summer. This result is surprising considering that the water column (0–100 m) productivity¹⁰ at these sites during winter ranged from 335 to 643 $\text{mg C m}^{-2} \text{ d}^{-1}$, which is about 2–3 times higher than that during April–May. The scavenging residence time of ^{234}Th during the summer and winter seasons is quite similar to that reported by Sarin *et al.*¹² at nearby locations. These results imply that the temporal variations in chemical scavenging rates of ^{234}Th in surface waters of the central Arabian Sea are not significantly influenced by the primary production and that the steady-state conditions assumed in the tracer scavenging model are nearly valid. Furthermore, our results from the Arabian Sea do not follow the linear trend between ^{234}Th scavenging rate and the primary productivity as reported by Coale and Bruland² for the Gulf of California.

The total ^{234}Th activity (dissolved + particulate) measured in the unfiltered samples also shows a pronounced deficiency with respect to ^{238}U in the upper 100 m water column (Figures 2 and 3). This deficiency of total ^{234}Th is attributed to its scavenging and subsequent downward export via settling particles. The mean residence time of ^{234}Th , with respect to its decay and removal from the water column, is about 100 days. Below 100 m, the total activity of ^{234}Th at stations along the 64°E transect is close to secular equilibrium value (Figure 2). In

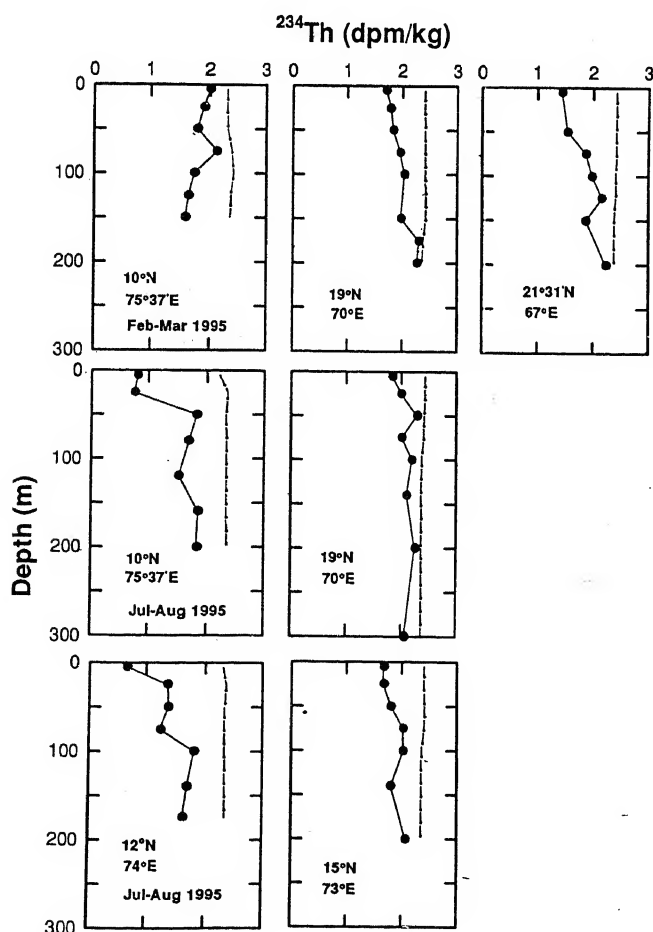


Figure 3. Activity of total ^{234}Th at coastal stations is plotted with respect to ^{238}U (dash line) indicating significant deficiency all through the water column.

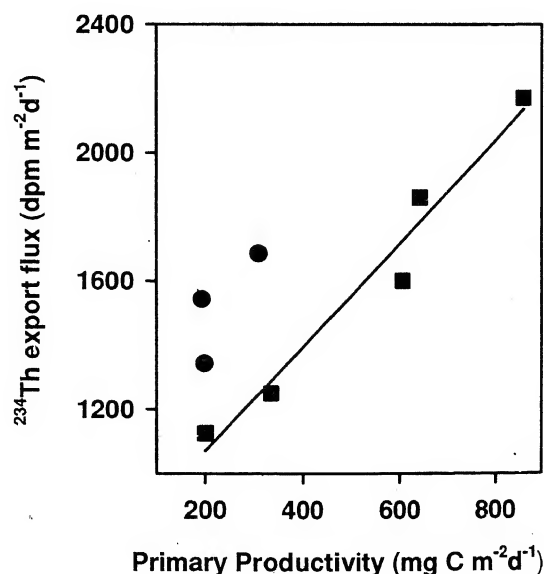


Figure 4. ^{234}Th export fluxes computed from the water column profiles as a function of primary production rate. Linear relation is seen for the data (filled squares) during winter (February–March). Data for summer (April–May) is shown by filled circles. Primary productivity data are from Bhattathiri *et al.*¹⁰

contrast, the total ^{234}Th activity at stations along the coast shows marked deficiency down to 200 m depth (Figure 3). One possible explanation for this is the higher particulate concentration in coastal waters and the rapid uptake of ^{234}Th near the sediment-water interface. At some of the coastal stations, this deficiency is relatively enhanced all through the water column during the monsoon period (July–August).

We have computed the removal rate of ^{234}Th from dissolved to particulate phases for two stations, viz. 22°N and 15°N (along the 64°E transect) based on the dissolved activities of ^{234}Th and ^{238}U measured during April–May and February–March (Figure 2). The average rate at which dissolved ^{234}Th is scavenged onto particles in the surface 100 m is estimated to be about $3400 \text{ dpm m}^{-2} \text{ d}^{-1}$. This rate for the central Arabian Sea is comparable to that reported for the California Current². The inventories of total ^{234}Th (dissolved + particulate) in the surface 100 m for the open ocean profiles, along the 64°E transect, are quite similar $(17 \pm 1)10^4 \text{ dpm m}^{-2}$ during the three sampling seasons (Table 1). The total ^{234}Th inventories vary from 12.4×10^4 to $19.7 \times 10^4 \text{ dpm m}^{-2}$ at the coastal stations (Table 1).

^{234}Th export

The measured deficiency of total ^{234}Th with respect to dissolved ^{238}U (Figures 2 and 3) has been modelled to derive the vertical export of particulate ^{234}Th from the upper 100 m water column (Table 1). The export fluxes at the coastal stations range from 915 to $2750 \text{ dpm m}^{-2} \text{ d}^{-1}$. At open ocean stations, ^{234}Th export rates show a north–south trend with higher values for the northernmost stations (Table 1). However, the derived export fluxes at an individual station are quite similar during

the summer (April–May) and winter (February–March) but tend to be somewhat higher during monsoon (July–August, Table 1).

The ^{234}Th export fluxes computed for the open ocean stations show a linear trend with the integrated column (0–100 m) primary productivity (Figure 4). This relationship, however, seems to be limited for the data from the same season. For example, the ^{234}Th export fluxes during February–March vary from 1125 to $2170 \text{ dpm m}^{-2} \text{ d}^{-1}$ while the column productivity varies from 200 to $860 \text{ mgC m}^{-2} \text{ d}^{-1}$. Although these export fluxes are somewhat identical with those during April–May (Table 1), the primary productivity is about a factor of three higher during the winter cruise (February–March 1995). These results suggest that the use of ^{234}Th as a survey tool for primary productivity needs close scrutiny.

Particle export

The particulate ^{234}Th export fluxes (at 100 m) based on water column deficiency are distinctly higher than those measured directly using floating sediment traps (Table 2), particularly in the samples collected during winter. During the winter season, the ^{234}Th flux measured by traps at 100 m is about a factor of four lower than that expected (assuming no lateral transport of particulate Th) from the water column deficiency (Table 2). Similar results are obtained based on the ^{210}Pb flux measured in the trap material and that expected from the atmospheric fallout¹³. This suggests that the shallow traps underestimate the particle export flux and the carbon export fluxes determined using them, without appropriate corrections for trapping efficiency, are likely to be underestimated. Similar observations were made by Bueseler *et al.*⁴ during the North Atlantic Bloom Experiment.

Table 1. Spatial and temporal variations in the inventory of total ^{234}Th (dissolved + particulate) and Th export fluxes at 100 m in the Arabian Sea

Location	Inventory (10^4 dpm m^{-2})			Export flux* ($\text{dpm m}^{-2} \text{ d}^{-1}$)		
	April–May 1994	February–March 1995	July–August 1995	April–May 1994	February–March 1995	July–August 1995
<i>Open ocean stations</i>						
$21^\circ 31'\text{N}$, 67°E	–	15.4	–	–	2170	–
$21^\circ 30'\text{N}$, 64°E	17.4	17	–	1685	1860	–
15°N , 64°E	17.9	17.4	15.7	1545	1600	2045
11°N , 64°E	18.1	18.4	17.3	1345	1250	1620
<i>Coastal stations</i>						
10°N , $75^\circ 37'\text{E}$	–	18.2	13.1	–	1125	2535
12°N , 74°E	–	–	12.4	–	–	2750
15°N , 73°E	–	–	17.4	–	–	1540
19°N , 70°E	–	17.3	19.7	–	1715	915

*Calculated based on the observed ^{234}Th : ^{238}U disequilibrium.

For deriving the particle export rates (Table 2), we have corrected for the trapping efficiency by assuming it to be the ratio of the measured ^{234}Th flux in the traps to that expected from its water column deficiency. It is also implicit here that the measured ^{234}Th flux in the traps deployed over 3–5 days is representative of the site.

Analogous calculations for the total material fluxes, from the upper 100 m column, have been made for other sampling sites in the Arabian Sea (Table 3). The particle export fluxes, corrected for trapping efficiency, range from 1890 to 3280 $\text{mg m}^{-2} \text{d}^{-1}$ during winter and are about a factor of 2 to 3 higher than those during summer. In each case, particle fluxes are obtained by dividing the estimated ^{234}Th export (Table 1) by ^{234}Th specific activity (dpm/g) measured in the traps (Table 2). The measured ^{234}Th activity in the trap material during April–May and February–March is 1856 and 662 dpm/g, respectively, and it is assumed to be rep-

resentative of all the sites during the respective season. A decrease in the specific ^{234}Th activity by a factor of 3 during winter could be related to the dilution of trap material by the increased abundance of particles/aggregates. This is in agreement with the measured $\text{C}/^{234}\text{Th}$ ratio during February–March (Table 2) when the column productivity is 2–3 times higher than that during April–May. Buesseler *et al.*⁴ have suggested that the temporal changes in $\text{C}/^{234}\text{Th}$ could occur as a function of local primary production and export balance. Relatively high $\text{C}/^{234}\text{Th}$ ratios were observed during bloom conditions in the North Atlantic⁴. We observe an increase in the $\text{C}/^{234}\text{Th}$ ratio from 0.24 during summer to 0.52 (mg/dpm) during winter suggesting that an enhanced vertical transport of sinking particles are ^{234}Th deficit. The organic carbon to total mass ratio in the traps is somewhat comparable between summer and winter, 0.43 and 0.35 respectively. This observation further reinforces the view that the dilution of trap material is most likely the cause

Table 2. Measured and estimated fluxes at sediment trap sites in the Arabian Sea

Location	Depth (m)	²³⁴ Th export (dpm m ⁻² d ⁻¹)		Particle export (mg m ⁻² d ⁻¹)		C/ ²³⁴ Th (mg dpm ⁻¹)	Carbon export (mgC m ⁻² d ⁻¹)	
		(1)	(2) [Ⓔ]	(1)	(2) [Ⓔ]	(1)	(1)	(2) [Ⓔ]
<i>April–May 1994</i>								
15°N, 64°E	100	1225	1545	660	830	0.24	290	371
<i>February–March 1995</i>								
21°30'N, 64°E	130	482	1860	728	2810	0.52	251	986

(1) Measured in the sediment trap material.

(2) Estimated based on water column profiles.

[®] From Table 1.

[§] Corrected for the trapping efficiency computed as the ratio of ^{234}Th flux calculated from the water column deficiency to that measured in the traps.

[£] Derived from $\text{C}/^{234}\text{Th}$ ratio in the trap and calculated ^{234}Th export.

Table 3. Export fluxes of ^{234}Th , settling particles and carbon at 100 m in the central Arabian Sea

Location	Column productivity [®] ($\text{mgC m}^{-2} \text{d}^{-1}$)	^{234}Th export [§] ($\text{dpm m}^{-2} \text{d}^{-1}$)	Particle export* ($\text{mg m}^{-2} \text{d}^{-1}$)	Carbon export* ($\text{mgC m}^{-2} \text{d}^{-1}$)
<i>April–May 1994</i>				
22°N, 64°E	310 [†]	1685	910	404
15°N, 64°E	193	1545	830	371
11°N, 64°E	199	1345	725	322
<i>February–March 1995</i>				
21°30'N, 67°E	860	2170	3280	1150
21°30'N, 64°E	643	1860	2810	986
15°N, 64°E	606	1600	2420	848
11°N, 64°E	335	1250	1890	662
10°N, 75°37'E	200	1125	—	—

[®] Data from Bhattathiri *et al.* (this issue).

[§] From Table 1.

* Obtained by dividing estimated ^{234}Th export by ^{234}Th specific activity (dpm/g). The ^{234}Th activity in the trap material during April–May and February–March is 1856 and 662 dpm/g, respectively. This specific activity is taken to be the same for all locations during the respective season.

[†] Derived from $\text{C}/^{234}\text{Th}$ ratio and ^{234}Th export.

[‡] Measured at 22°N, 68°E.

for the decrease in particulate ^{234}Th activity during winter.

As proposed by Eppley¹⁴, the other purpose of studying ^{234}Th – ^{238}U disequilibrium and particle export of ^{234}Th , in the upper 100m, is to relate it to carbon export flux out of the euphotic zone. The C export flux has been computed as a product of the ^{234}Th export flux (Table 3) and C/ ^{234}Th ratio for the corresponding period (Table 2). The calculated flux is distinctly higher than the measured C export at the trap site during winter (Table 2), suggesting that an appropriate correction for particle trapping efficiency is needed for the reliable estimates of C using floating sediment traps. In general, estimated C export fluxes at all stations are higher than the column primary productivity (Table 3). There could be two explanations for the estimated C fluxes being higher than the primary productivity: (i) there is an additional source of carbon via the microbial loop and that the C/ ^{234}Th measured in the trap material (collected over 3–5 days period) is not representative of the site and water column POC inventory, or (ii) there is a phase lag between primary production and flux out of the euphotic zone. We propose the first explanation to be more dominant as there is an evidence for active microbial pathway¹⁵. Also, C/ ^{234}Th ratio of the settling particles in the trap may not be appropriate for estimating organic C export. Indeed the direct measurement of C flux in the traps during April–May is also higher than the primary production (Table 2). These observations raise the question of differential cycling of the various components of particulate matter and ^{234}Th in the upper 100 m of the Arabian Sea.

Conclusions

The ^{234}Th : ^{238}U disequilibrium in the upper 100 m has been used to derive the chemical scavenging rates and the export fluxes of ^{234}Th and settling particles in the Arabian Sea. In the open ocean, the residence time of dissolved ^{234}Th with respect to its removal onto particles is ~ 30 days. We find that the estimated vertical export fluxes of particulate ^{234}Th (at the respective sites) are similar between summer and winter and that the export fluxes are higher in general over the regions characterized

by higher rates of column productivity. The ^{234}Th flux data are used to derive the fluxes of particulate matter and carbon at 100 m. The computed fluxes of particles are significantly higher than those measured using free-floating sediment traps. The C export fluxes are also higher than the column primary productivity suggesting that the direct comparison of these fluxes with independent estimates of new production is needed. The future studies should aim towards understanding the relationship between C and ^{234}Th on sinking particles and dynamics of carbon cycling in the Arabian Sea.

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Distribution of nitrous oxide and methane in the Arabian Sea

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Measurements of the two important biogenic gases N_2O and CH_4 have been made both in the water column and in air of the Arabian sea during April–May 1994 and February–March 1995, as part of the JGOFS (India) programme. The average abundances of N_2O and CH_4 in the air are 313 ± 7 ppbv and 1.69 ± 0.05 ppmv respectively. During both the periods, the vertical profiles of N_2O in the water column show a double peak structure with the dominant peak in the 500–1000 m depth region with concentrations of 70–80 nmol. The entire water column is supersaturated with respect to N_2O and in the major peak region it is supersaturated by as much as 600–800%, suggesting this region of the Arabian sea to be an important reservoir of N_2O . During February–March 1995, sea–air flux of N_2O is calculated to be about $0.26 \text{ pg cm}^{-2} \text{ s}^{-1}$, while the flux calculated for April–May 1994 is not significantly different from zero. Methane concentrations were measured only down to 400 m depth. Its distribution shows a peak concentration of 6–8 nmol in the 100–200 m depth region. Supersaturation (up to 200–400% at the peak) is found for CH_4 in most of the profiles. Average sea–air flux of methane during April–May 1994 and February–March 1995 is 0.0001 and $0.033 \text{ pg cm}^{-2} \text{ s}^{-1}$, respectively.

Nitrous oxide (N_2O) and methane (CH_4) are important minor constituents of the earth's atmosphere. Recently, N_2O is being studied with growing interest due to its greenhouse warming potential¹ and as a source of NO radicals in the stratosphere which participates in the catalytic ozone depleting reactions². N_2O emanates from the earth's biosphere due to various natural and anthropogenic activities, the latter include biomass burning, fertilizer use and land use. It is estimated that the oceans also contribute significantly (20–30%) to the global budget¹ of N_2O . Recent studies in the Arabian Sea show a significant ocean to atmosphere flux of N_2O in spite of its relatively small area (0.43%) of the world oceans^{3,4}.

Methane plays an important role in controlling the abundance of atmospheric water vapour and hydroxyl radical⁵, and it also takes part in tropospheric ozone chemistry and greenhouse warming. Major sources of methane to the atmosphere are wetlands, paddy fields and enteric fermentation¹. In general, oceans are a relatively minor source of methane. Sub-surface ocean water has been found to be supersaturated at different loca-

tions^{6–8}. Under the Joint Global Ocean Flux Study (India) programme, these trace gases have been measured both in air and in the water column during April–May 1994 (cruise No. SK-91) and February–March 1995 (cruise No. SK-99) in the north-east Arabian Sea (11°N–22.5°N, 64°E–74°E). In this article we describe the results and provide estimates of the sea–air fluxes of N_2O and CH_4 .

Experimental procedure

Measurements of N_2O and CH_4 were made using two gas chromatographs (GCs) installed on board the ORV *Sagar Kanya*. N_2O was measured using a Varian VISTA series gas chromatograph equipped with an electron capture detector (GC-ECD). The ECD is widely used to measure nitrous oxide and various kinds of halo-carbons^{9–11} due to its high sensitivity and sufficient linear response¹². A $5 \text{ m} \times 0.32 \text{ cm}$ stainless steel column packed with Porapak-Q (80/100 mesh), maintained at 40°C, was used for N_2O analysis. A Shimadzu GC equipped with a flame ionization detector was used for measuring CH_4 . A stainless steel column of dimension $5 \text{ m} \times 0.32 \text{ cm}$, packed with molecular sieve-13X maintained at 60°C was used. Atmospheric pressure inside the laboratory was recorded using an MKS baratron sensor for pressure correction.

Air samples were collected in 50 ml B–D plastic syringes from the bow of the ship while cruising (i.e. between stations). Samples were injected into the GC column after passing through a moisture trap and using a 4 ml loop connected to a 6 port valve. Both these were flushed with the same sample before final injection. Each sample was run 2–3 times to obtain representative concentrations. Sea-water samples were collected from various depths using 1.7 l Niskin bottles during SK-91 cruise and 30 l Go-Flo bottles fitted to a CTD Rosette during the SK-99 cruise. During April–May 1994, water samples were collected down to a depth of 3000 m at several stations, but during February–March 1995 the depth of sampling was restricted to a maximum of 1000 m. Samples were drawn from these bottles into 50 ml glass bottles and stored in a refrigerator until analysis. Most of the sample analyses were completed within 24 hours of collection using standard techniques. 25 ml of water sample and 25 ml pure He were equili-

brated in a B-D syringe for about 5 minutes at room temperature. 4 ml of this equilibrated He was injected on to the GC column after passing through a moisture trap filled with $\text{Mg}(\text{ClO}_4)_2$. A similar procedure was followed for CH_4 analyses except that in this case 30 ml of sea water was equilibrated with 20 ml of pure He. Duplicate samples were analysed periodically to check for reproducibility of results and to evaluate the precision of measurements. The replicate measurements were found to be within 10%. A 50 l cylinder filled with air (from PRL, Ahmedabad) was used as the laboratory standard which was analysed frequently to check for stability of the instruments. This standard was calibrated for N_2O and CH_4 using standard mixtures of known concentrations. The concentrations of N_2O and CH_4 in the standard air were estimated to be 313 ppbv and 1.94 ppmv respectively.

Concentrations and fluxes

Concentrations of N_2O and CH_4 in air are calculated by comparing the GC response with the response obtained from the laboratory standard. The mole concentrations of dissolved gases in sea water are calculated based on the principle of multiple phase equilibrium as given by McAuliffe¹³. For example, the concentration of N_2O in sea water in nmol can be expressed as

$$\text{N}_2\text{O (nmol)} = 0.0409 \times \frac{1}{H_x} \times P_L \times \frac{298}{T_L} \times \text{N}_2\text{O (ppbv)}, \quad (1)$$

where H_x is Henry's law constant, which is a function of temperature and salinity, P_L and T_L are the laboratory pressure (atm) and temperature (K) respectively, N_2O (ppbv) is the mixing ratio of nitrous oxide in the zeroth order equilibrated air with sea water, and the constant (0.0409) is the conversion factor from mixing ratio (ppbv) to concentration (nmol) at STP. This concentration is defined as observed concentration (C_{obs}). A similar equation is used for calculating the methane concentration.

Saturation concentrations (C_{sat}) of the respective gases in water were calculated by multiplying their partial pressure in the ambient marine air with their solubility in sea water at the appropriate temperature and salinity. The solubility of N_2O and CH_4 in sea water was calculated by using the relations of Weiss and Price¹⁴ and Atkinson and Richards¹⁵, respectively. The flux (F) in $\text{nmol cm}^{-2} \text{s}^{-1}$ between air and water was calculated by using the wind speed dependent transfer velocities¹⁶ and can be expressed as

$$F = k \times \Delta C, \quad (2)$$

where ΔC is the difference in concentrations ($= C_{\text{obs}} - C_{\text{sat}}$) and k is the gas transfer velocity in cm h^{-1} . The value of k depends on wind velocity and is evaluated using one of the following equations

$$k = 0.17 U_{10} (660/S_c)^{2/3} \quad U_{10} \leq 3.6$$

$$k = (2.85 U_{10} - 9.65) (660/S_c)^{1/2} \quad 3.6 < U_{10} \leq 13 \quad (3)$$

$$k = (5.9 U_{10} - 49.3) (660/S_c)^{1/2} \quad U_{10} > 13.$$

U_{10} is the wind speed (m s^{-1}) at 10 m height from the sea surface, S_c is the Schmidt number of the respective solute gas, calculated using the expressions given by Wanninkhof¹⁷. Note that the constant 660 which is the Schmidt number of CO_2 at 20°C in sea water has been used instead of 600, which is for the freshwater, as proposed in a more recent analysis¹⁷. A negative flux value suggests ocean as a sink for the solute gas whereas a positive value implies ocean to be a source to the atmosphere.

Results

Latitudinal distributions in the concentrations of N_2O measured in air during SK-91 and SK-99 along 64°E are shown in Figure 1. The data show a significant scatter, from about 300 to 335 ppbv without any systematic trend. The average concentration was found to be 313 ± 7 (1σ ppbv). The spread ($\pm 1\sigma$) is similar to analytical precision (2%), however, the error in absolute concentrations could be larger ($\pm 5\%$) due to calibration uncertainties. Figure 2 shows typical distributions of N_2O in sea water down to 3000 m depth during the SK-91 cruise. The concentration of N_2O increases very sharply from about 6 nmol in the surface water to about 50 nmol at around 150–200 m depth. At depths between 200 and 300 m, there is a decrease in the concentration particularly in the northern latitudes. N_2O concentration increases again to values as high as 60–80 nmol at around 700 m depth; below which it decreases with depth to a value of 20 nmol at around 3000 m. Figure 2 also shows the expected concentrations of N_2O for 100% saturation (dotted line). It is evident from Figure 2 that N_2O is at or above saturation throughout the water column. At the major peak (500–1000 m), supersaturation is in the range of 600% to 800%. Even at 3000 m depth the value ranges between 130% and 160%. Similar general features in the vertical distribution of N_2O have also been observed in the profiles measured during subsequent cruise (SK-99).

Figure 3 shows a contour plot of N_2O concentrations for the latitude region of 11 to 22°N measured during SK-91. The distribution shows three high N_2O concentration regions centered at 13°N , 17°N and 22°N with

values as high as 77 nmol, with the maximum values occurring at deeper depths in higher latitudes; at 13°N it is located around 500 m depth while at 22°N it is at ~900 m depth. Along 64°E longitude the shallower peak is more intense towards northern latitudes. Although

somewhat similar features have been reported during measurements made in December 1988 in the Arabian sea along 67°E longitudinal transect⁴, such detailed structures have not been observed. A comparison of N₂O concentrations in sea water obtained during the two

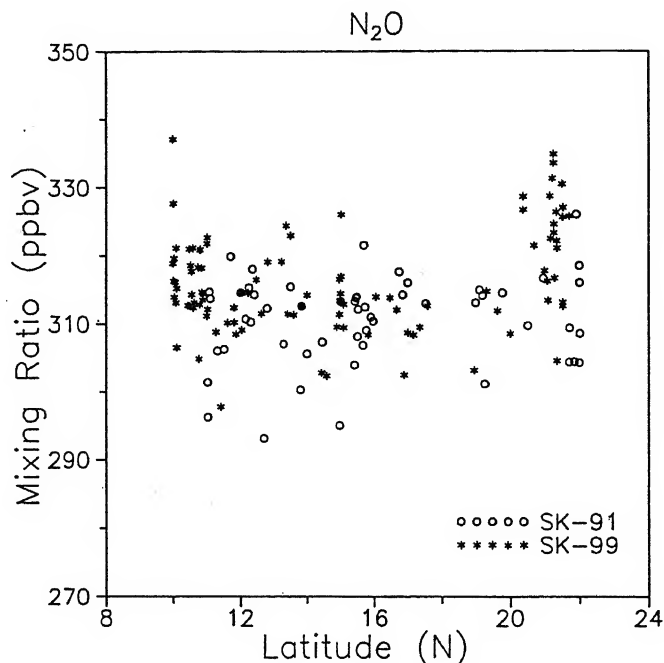


Figure 1. Distribution of nitrous oxide in the Arabian Sea air measured along the cruise track during April–May 1994 (SK-91) and February–March 1995 (SK-99).

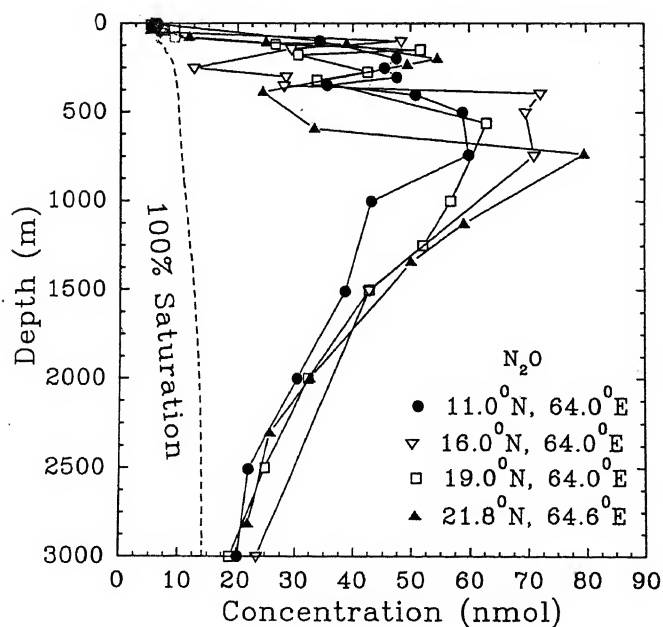


Figure 2. Examples of the vertical distribution of N₂O in the water column along 64°E longitude.

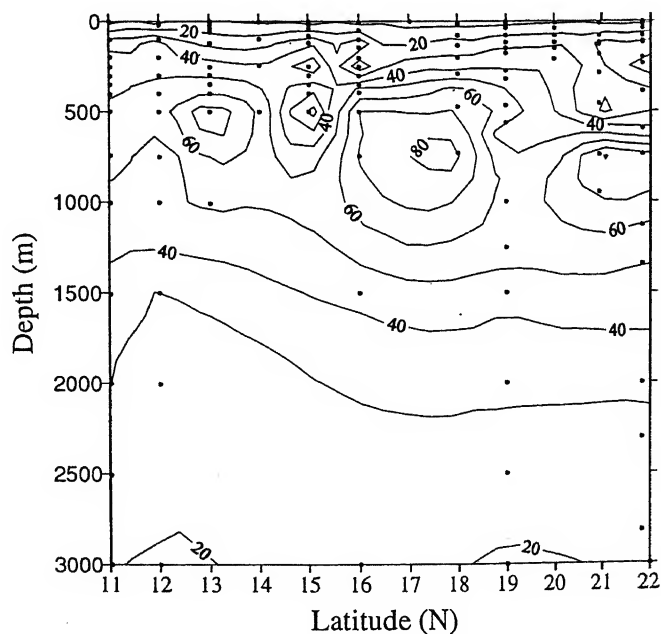


Figure 3. Distribution of nitrous oxide (nmol) showing the latitudinal variability down to 3000 m depth observed during SK-91 cruise.

cruises is shown in Figures 4a and 4b. A broad maximum around 17–18°N latitude and 800 m depth was seen during both the cruises. However, the finer scale features in the shallow depths (0–600 m) are different. The two low N_2O regions which were located around 500 m depth during SK-91 were observed at a shallower depth (300–400 m) during SK-99. During both these cruises higher concentrations of N_2O were observed, centered around 17°N, between these two minima.

Figure 5 shows methane concentrations in air during SK-91 and SK-99 cruises. The results show marginally higher CH_4 concentrations (1.70) during SK-91 than those (1.66) during SK-99, with an average value of 1.69 ± 0.05 ppmv for the combined data of both the cruises. The spread is within the range of analytical

errors. Measurements of methane in the water column were made for the north of 16°N of the cruise track during SK-91 and along the complete track during SK-99. Typical concentration profiles observed during SK-99 are shown in Figure 6. The concentration of methane in the surface water was observed to be about 3 nmol, increasing to value of 4–9 nmol at around 100–200 m. Below this depth, its concentration decreased gradually to 2–4 nmol. Methane is supersaturated at almost all depths from the surface down to 400 m depth. Supersaturation at the maximum concentration varied from 200 to 400%.

There is a large variability in methane distribution from open ocean to the coast. In the shallow coastal waters, the maximum CH_4 concentration was higher. The vertical profiles of CH_4 as depicted in Figure 6 for 16°N and 18°N represent features of the open ocean, the one at 22°N shows characteristics of a coastal region. Prior to these studies, the only other reported measurements of CH_4 in the Arabian Sea were due to Owens *et al.*⁷ These results also showed a supersaturation of CH_4 , however, the nature of the CH_4 profiles, particularly the depth of CH_4 maximum differs in these two studies. Figures 7a and 7b show the spatial and depth variations of methane concentration along the 64°E meridian. The major peak in methane was observed at 19°N around 150 m depth during SK-91 whereas the peak was found to be at shallower depth (~125 m) during the later cruise (SK-99). It can also be seen that the depth of

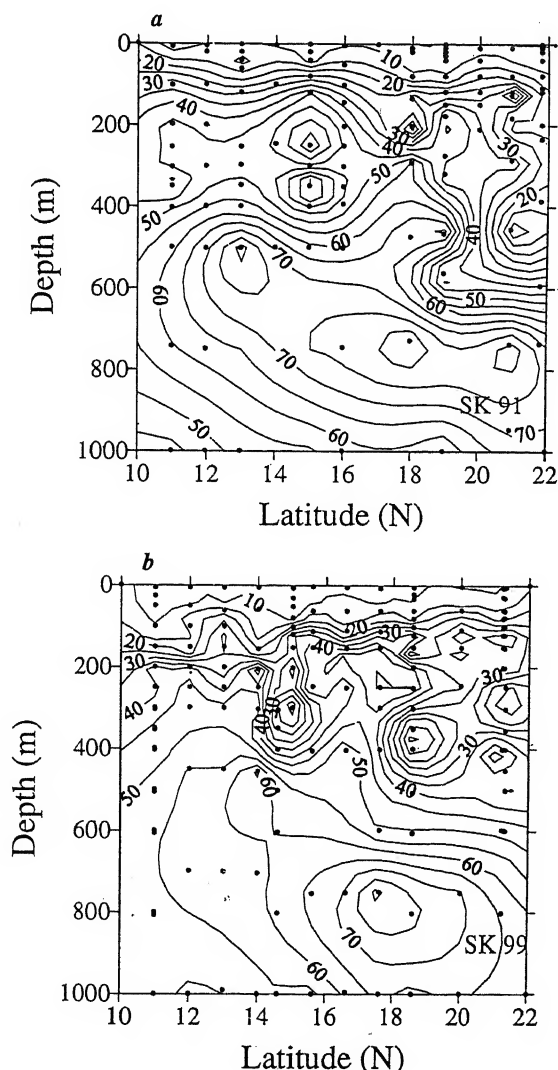


Figure 4. Distribution of nitrous oxide (nmol) showing latitudinal distribution down to 1000 m depth for SK-91 (a) and for SK-99 (b). All contours are shown at an interval of 5 nmol.

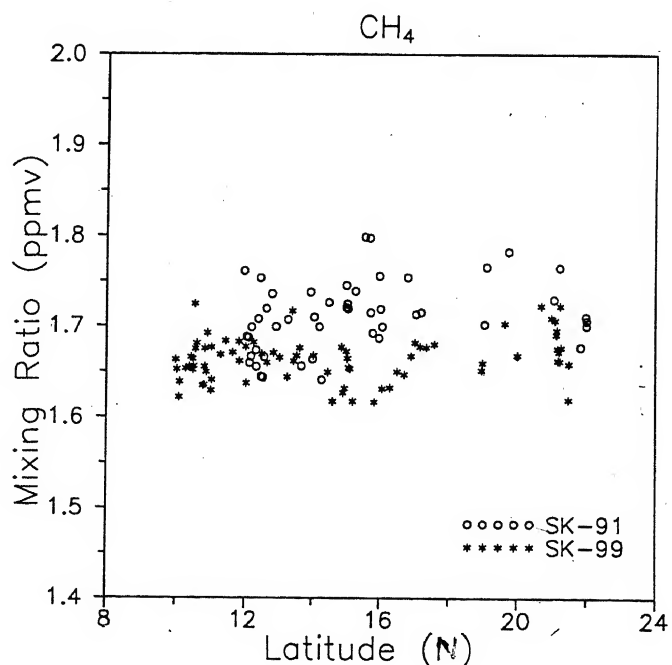


Figure 5. Mixing ratios of methane as observed in marine air during SK-91 and SK-99 cruises.

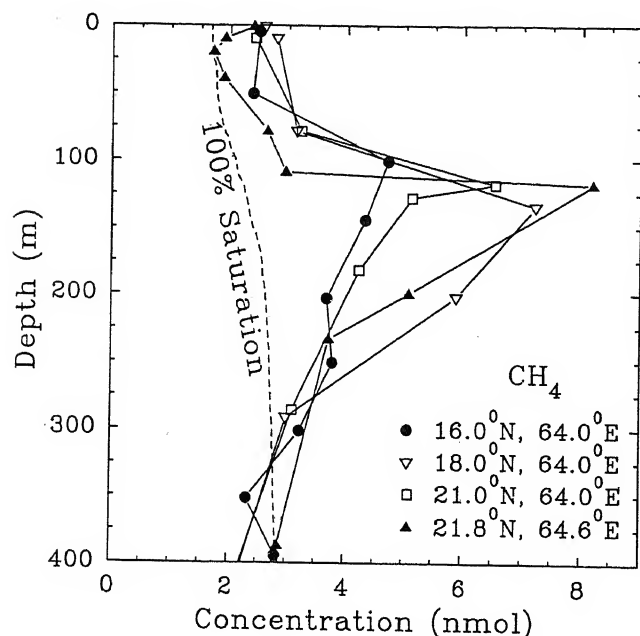


Figure 6. Examples of the vertical distribution of CH_4 (nmol) in the water column during SK-91 cruise.

methane maximum decreases with increase in latitude (Figure 7b). Thus at 11°N the methane maximum occurs at a depth of about 200 m, while at 19°N it is observed at around 125 m depth (Figure 7b).

The sea-air fluxes of CH_4 and N_2O have been computed using equations (2) and (3) described earlier. Fluxes from or into sea water depend upon the degree of saturation in surface water and the wind speed. There is no significant supersaturation of N_2O in the surface water during SK-91 (April–May 1994). The N_2O content in surface water during this period is within $\pm 10\%$ of the concentration expected from the partial pressure in air, however, an average flux of about $0.003 \text{ pg cm}^{-2} \text{ s}^{-1}$ can be calculated. The range of ΔC in surface waters during this cruise is within errors ($\pm 1\sigma$) of measured N_2O concentrations. However, during SK-99 (February–March 1995), there is a distinct supersaturation of N_2O in surface water, leading to an average ocean-air N_2O flux of about $0.26 \text{ pg cm}^{-2} \text{ s}^{-1}$. Our values are within the range of earlier reported values of $0.44 \pm 0.22 \text{ pg cm}^{-2} \text{ s}^{-1}$ (September–October, 1986)³ in north-west Arabian Sea and $0.23 \pm 0.1 \text{ pg cm}^{-2} \text{ s}^{-1}$ in the north-east Arabian Sea⁴.

The average methane flux from the surface water into the atmosphere was estimated to be about 0.0001 ± 0.006 (1σ) $\text{pg cm}^{-2} \text{ s}^{-1}$ during SK-91, while during SK-99 the average flux was found to be about $0.033 \pm 0.046 \text{ pg cm}^{-2} \text{ s}^{-1}$. Owens *et al.*⁷ measured methane fluxes in the range of 0.08 to $0.26 \text{ pg cm}^{-2} \text{ s}^{-1}$ (0.46 to $1.4 \text{ nmol cm}^{-2} \text{ day}$). Low values of both N_2O and CH_4 fluxes measured during April–May 1994 period are due to lesser degree

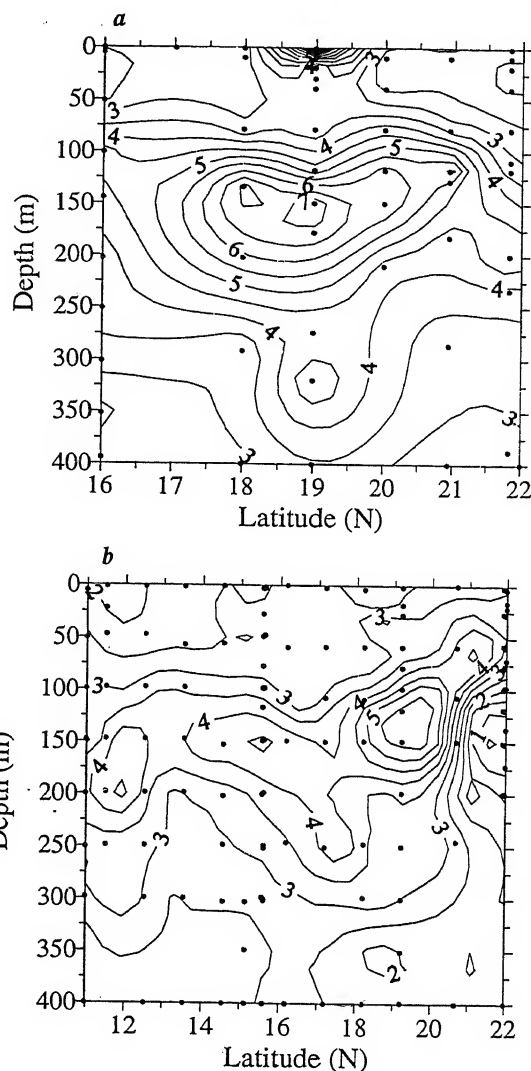


Figure 7. Latitudinal depth contour plots of methane (nmol) observed during SK-91 (a) and during SK-99 (b). Alternate contours are labelled.

of supersaturation and lower wind speed prevailing in the Arabian Sea.

Discussion

The distributions of N_2O with depth in water show two peaks. The shallow peak in the 150–200 m depth region is more pronounced in the northern region, beneath which is a concentration maximum in the 500–1000 m depth. Below this maximum the concentration gradually decreases with depth, down to 3000 m. The low concentrations between the two peaks coincide with the secondary nitrite maximum resulting from denitrification. In this region N_2O itself is reduced by the bacteria. Both nitrification and denitrification are considered to be the processes responsible for the production of N_2O .

in sea water^{4,9,10,18,19}. Besides the supersaturation of N₂O in the entire water column, large latitudinal and longitudinal variations were observed. During the SK-91 cruise (April–May 1994) there is no appreciable N₂O sea–air flux, whereas during SK-99 (February–March 1995) an average flux of about 0.26 pg cms is estimated. During SK-91, the sea surface conditions were more quiet and there was no clear supersaturation in surface water which lead to insignificant flux of this gas across the boundary. During SK-99 period, wind speeds were relatively higher and the waters were relatively more supersaturated. The mixed layer depth (MLD) was much deeper during SK-99, in the range of 70 to 120 m while during SK-91 it ranged between 20 and 35 m (ref. 20). A higher MLD is likely to give rise to higher concentration due to mixing with deeper N₂O and CH₄ rich water. The MLD was observed to be deepest at 16°N during SK-91 and at 17°N during SK-99 where high N₂O concentrations were observed to be protruding upwards. Compared to the results of present measurements Law and Owens³, and Naqvi and Noronha⁴ have observed significantly different fluxes of N₂O to the atmosphere in September–October 1986, and December 1988 respectively which, in general, suggest strong seasonal dependence of its fluxes.

Measurements of CH₄ are made for the first time in the north-eastern part of the Arabian Sea. These data show maximum concentrations in the region of 100 to 200 m depth. Owens *et al.*⁷ in their studies in western Arabian Sea observed the peak to be located at around 60–100 m depth. This difference is attributed to the deeper Chlorophyll *a* layer in the eastern region (~60 m)²¹ than in the western region (30–40 m)⁷ of the Arabian Sea. During both cruises the methane fluxes to the atmosphere have been observed, with higher fluxes in the SK-99 period. Owens *et al.* observed methane fluxes ranging from 0.08 to 0.26 pg cm⁻² s⁻¹ in the western Arabian Sea, considerably higher than those observed in other oceanic regions⁸ and this study.

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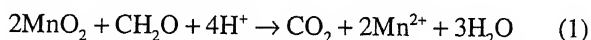
Manganese mobilization from the Western Continental margin of India

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The Western Continental margin of India (WCMI) exhibits distinct features with respect to vertical distribution of manganese in sediments. There is no significant manganese downcore variation in upper slope sediments whereas high concentration (by an order of magnitude relative to bottom sections) has been observed in top sediment cores for the lower slope region. Also, in the upper slope sediments, average manganese concentration is low by a factor of three compared to that (~1000 ppm) transported by the Narbada/Tapti river suspended particles. The low manganese concentration is attributed to its reductive mobilization. Typical value of manganese flux being mobilized into overlying seawater from reducing upper slope sediments covering an area of 5°Lat × 3°Long is calculated to be 3×10^{10} g/y, which is nearly 10% of dissolved manganese supply to the ocean by world rivers. Although calculated flux of manganese mobilized from upper slope sediments is not the highest among the values from other margin regions, yet this shows a potential source for dissolved manganese in seawater. Interestingly, the calculated carbon oxidation flux (based on manganese reduction rate) in the upper slope sediments is found to be less than 1% of primary productivity and, thus it provides a limiting value of organic matter combustion. Contrary to upper slope sediments, there is no significant supply of soluble manganese to overlying seawater, which is expected due to prevailing oxidizing conditions at the sediment-water interface in the lower slope region.

The biogeochemical cycle of Mn in marine environment plays an important role in regulating trace metal distribution, as manganese oxides are potential scavengers of several trace metals¹. In sediments, Mn oxides undergo reduction to soluble Mn^{2+} during anaerobic decomposition of organic matter^{2,3}.



Therefore, in reducing conditions Mn is released from sediments to pore waters and is subsequently transported to overlying seawater by advective-diffusion processes either in part or completely depending on the redox conditions near the sediment-water interface. In this mobilization process, only some fraction of Mn (reactive component) associated with continental/riverine sediment

inputs is mobilized, leaving behind some fraction in the sediments. At some places of margin regions⁴, high concentration of Mn is reported in top oxidizing sedimentary layer as it acts as a cap for Mn^{2+} escape from below the sediments. These are the major pathways of Mn cycling in margin regions. Release of Mn^{2+} from reducing sediments and thereafter supply to overlying seawater generally takes place if manganese oxidation rate at sediment-water interface is slower than that of its reduction rate in the sediment below the surface⁵⁻⁷. Factors controlling release of Mn^{2+} from sediments to overlying seawater and its distribution in various layers within the sediments include not only the bottom water O_2 concentration⁸⁻⁹, temperature and organic input^{3,7} but also depend on precipitation of authigenic carbonate mineral phases such as calcite¹⁰⁻¹² and Mn-carbonate¹³⁻¹⁴ from the pore waters. Carbonate mineral phases in the sediment can act as a sink for soluble Mn^{2+} . Also, sediments characterized by bioturbation show increased supply rate of Mn^{2+} to the overlying seawater^{5-9,15}.

Earlier studies in sediments from the Western Continental margins of India (WCMI) have shown depletion of Mn along the open shelf and slope regions^{16,17}. More recently, Somayajulu *et al.*¹⁸ attributed variations in the concentrations of manganese in sediments from the shelf and slope regions of WCMI to be due to differences in prevailing redox conditions. This paper synthesizes available data on the manganese distribution in WCMI sediments to obtain constraints on its fluxes to the overlying water column and its role in mineralization of organic matter.

Materials and methods

Five spade sediment cores (50 × 30 × 20 cm) from the WCMI region (Figure 1) were collected during December 1988. Of these, three cores (L-8, J-7 and I-5) are from the upper slope region (water depth ranging from 280 to 350 m) where the core of denitrification layer (centered at ~300 m depth)¹⁹ meets the sediment-water interface. The remaining two cores, viz. K-11 and M-12 are from the lower slope region where water depth is ~2500 m. Immediately after collection, sub-cores of ~5 cm diameter and ~30 cm in length were taken from these spade cores. The sub-cores were sampled at 1–2 cm

depth intervals for CaCO_3 , loss on ignition (LOI), major, trace elements and radionuclide measurements. Intense H_2S smell was felt during sectioning of the cores L-8 and J-7 at about ~ 5 cm below the surface, indicating strong anoxic conditions prevailing near the surface of these cores. On the contrary, no H_2S smell was felt from any layer while sectioning the cores from the lower slope region. Chronology of these cores was determined mainly by the $^{210}\text{Pb}_{\text{excess}}$ and ^{137}Cs methods^{17,18}.

Results and discussion

It is well documented that in anoxic marine sediments, where sediment accumulation is relatively high, manganese is mobilized as Mn(II) from the solid phase to solution²⁰⁻²². In margin areas, the primary source of Mn to sediments is land-derived. Hence, one of the approaches to obtain information on Mn mobilization is to compare the abundance of Mn in margin sediments with that in fluvial sediments. Such study has an important implication to elucidate high content of manganese in deep ocean sediments. In fact, early sediment diagenesis leading to recycling of manganese from near-shore region is considered to be an important 'source' for high manganese concentration in deep ocean reservoir. For instance, suggestion has been made by several workers that a 'source' of Mn to the central North Pacific may be from reduced continental shelf sediments²³⁻²⁷. In the following, manganese mobilization that occurs across the transect encompassing both anoxic (upper slope) and oxic (lower slope) sedimentary environments of the Arabian sea are discussed.

Upper slope sediments (L-8, J-7 and I-5)

The depth profiles of manganese in these sediments are

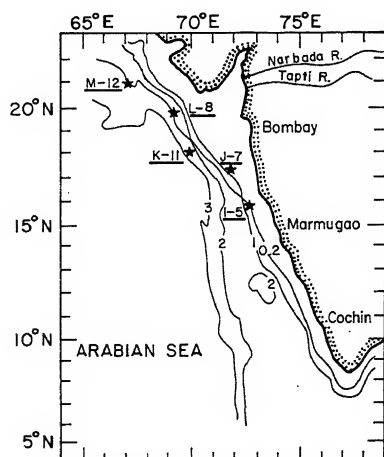


Figure 1. Map showing core locations (*) along the WCMI with water-depth contours in km.

presented in Table 1 and Figure 2a, though they have been published earlier^{17,18}. The manganese concentration in all these cores is generally low (~ 285–350 ppm, Table 1a) compared to that in the Narbada and Tapi river suspended particles (~ 1000 ppm)¹⁶. The low Mn concentration in these sediment cores relative to the source material is attributed to the reductive mobilization of Mn from the sedimentary column. As the upper slope sediments are from shallow water depths, manganese mobilization from fluvial sediments and particulates during their transit through anoxic denitrification water layer is expected to be minimal. With this assumption, a simple calculation is done to estimate the flux of Mn being mobilized from the upper slope reducing sediments.

The flux of manganese mobilized from sedimentary column, F_{Mn} ($\mu\text{g}/\text{cm}^2 \cdot \text{y}$), can be obtained using the relation

$$F_{\text{Mn}} = S \cdot \rho (C_r - C_s), \quad (2)$$

where S is the sediment accumulation rate (cm/y), ρ the in-situ density (g/cm^3) of the sediment, C_r and C_s are the manganese concentrations in river particles (~ 1000 ppm)¹⁶ and in upper slope sediments. The value of F_{Mn} for the three cores L-8, J-7 and I-5 is calculated to be 7.4, 68.7 and 17 $\mu\text{g}/\text{cm}^2 \cdot \text{y}$ respectively using their column average Mn concentration (Table 1a), density (0.44, 0.58 and 0.61 g/cm^3) (refs 28, 29) and rate of sediment accumulation (0.025, 0.183 and 0.039 cm/y)^{18,28}. In this method of flux calculation for manganese, there is some degree of uncertainty associated with the assumption of constant Mn concentration (~ 1000 ppm) in river-borne suspended sediments. Earlier study has shown that Narbada and Tapi rivers which drain into the study region show a slight seasonal variation in manganese concentration of suspended particles (Narbada: 1000–

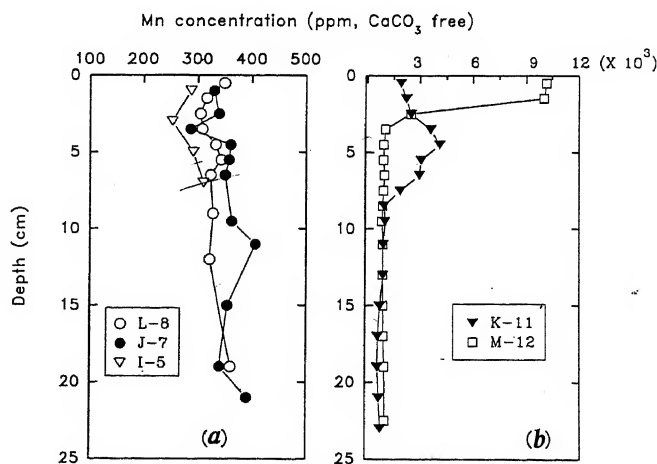


Figure 2. Downcore variation of Mn abundance in the lower slope sediments. The Mn distribution in the lower slope sediments shows a distinct structure with high values near the sediment–water interface.

Table 1a. Mn concentration in the upper slope sediments on CaCO₃ free basis

Core: L-8		Core: J-7		Core: I-5	
Depth (cm)	Mn (ppm)	Depth (cm)	Mn (ppm)	Depth (cm)	Mn (ppm)
(0-1)	349	(0-2)	330	(0-2)	287
(1-2)	316	(2-3)	339	(2-4)	253
(2-3)	303	(3-4)	286	(4-6)	291
(3-4)	308	(4-5)	360	(4-6)R	273
(3-4)R	314	(5-6)	357	(6-8)	310
(4-5)	333	(6-7)	349		
(5-6)	343	(9-10)	362		
(6-7)	323	(10-12)	406		
(8-10)	328	(14-16)	354		
(10-14)	321	(18-20)	340		
(18-20)	359	(20-22)	390		
$\overline{\text{Mn}} =$	327*	$\overline{\text{Mn}} =$	352*	$\overline{\text{Mn}} =$	283*

*Concentration averaged over entire core length.

R denotes replicate analysis.

Table 1b. Mn concentration in the lower slope sediments on CaCO₃ free basis

Core: K-11		Core: M-12	
Depth (cm)	Mn (ppm)	Depth (cm)	Mn (ppm)
(0-1)	1903	(0-1)	10161
(1-2)	2211	(1-2)	10017
(2-3)	2479	(2-3)	2470
(3-4)	3581	(3-4)	997
(4-5)	4092	(4-5)	908
(5-6)	3052	(4-5)R	930
(6-7)	2952	(5-6)	897
(7-8)	1862	(6-7)	947
(7-8)R	1841	(7-8)	889
(8-9)	922	(8-9)	847
(9-10)	975	(9-10)	810
(10-12)	881	(10-12)	865
(12-14)	865	(12-14)	864
(12-14)R	839	(14-16)	884
(14-16)	692	(16-18)	887
(16-18)	574	(18-20)	944
(18-20)	564	(20-25)	958
(20-22)	631		
(22-24)	711		
Mn^*	1340	Mn^*	1270

*Mn indicates median value of concentration over entire core length.
R denotes replicate analysis.

1300 ppm, Tapti: 1200–1300 ppm)¹⁶. With this uncertainty, a typical value of manganese flux from upper slope reducing sediments to overlying seawater can be taken as $21 \mu\text{g}/\text{cm}^2 \cdot \text{y}$, which is the geometric mean of Mn fluxes for three cores. If this calculation is extended to an area of $5^\circ\text{Lat.} \times 3^\circ\text{Long.}$, bounded by these cores, the flux of Mn would be $\sim 3.0 \times 10^{10} \text{ g/y}$ which is nearly

10% of the dissolved Mn ($\sim 3 \times 10^{11} \text{ g/y}$) transported by world rivers to ocean²⁷. Such a high flux of Mn out of sediments from the upper slope region of the Arabian Sea emphasizes the importance of mobilization of Mn in the margin region. It is likely that manganese released from these sediments is a source of dissolved Mn maxima reported in the Arabian Sea intermediate waters³⁰⁻³¹. In order to evaluate the importance of manganese mobilization from reducing sediments as 'source' for seawater, benthic chamber study is necessitated³².

Table 2 compares the flux of Mn released from river-borne particles from the upper slope sediments of the Arabian Sea with the fluxes reported from other coastal margin areas of the world ocean. The flux of Mn solubilized is generally related to rates of sedimentation, biological productivity and also to the particle mixing by bioturbation⁴. Sundby and Silverberg³³ have studied manganese cycling in Laurentian trough (water depth, 300–400 m) and applied a diagenetic model and mass-balance calculations to derive the Mn flux. They observed a net flux of $140\text{--}180 \mu\text{g}/\text{cm}^2 \cdot \text{y}$ (Table 2). The magnitude of particle mixing by bioturbation considerably influences the rate of Mn cycling both in the sediment core (internal cycling) and the fraction mobilizing to overlying seawater. Trefry and Presley³⁴ have interpreted the deficiency of Mn in the Mississippi delta sediments, compared to that in the river particulates, as due to its mobilization under reducing conditions. Using the concentration gradient of Mn in sediment pore waters, they calculated the outgoing diffusive manganese flux from sediments (water depth < 100 m) in the range of $30\text{--}850 \mu\text{g}/\text{cm}^2 \cdot \text{y}$ (Table 2). Johnson *et al.*³² have

Table 2. The benthic manganese flux from margin sediments

Margin sites	Mn flux ($\mu\text{g}/\text{cm}^2 \cdot \text{y}$)	Reference
WCMI (upper slope)	~ 35	This study,
Central California coast line	~ 10	Johnson <i>et al.</i> ¹⁴
Gulf of St. Lawrence	~ 140–180	Sundby and Silverberg ³³
Mississippi Delta	~ 30–850	Trefry and Presley ³⁴
Eastern Bering shelf	~ 1	Heggie <i>et al.</i>

reported an average benthic Mn flux of $\sim 10 \mu\text{g}/\text{cm}^2 \cdot \text{y}$ from the central California continental margin.

In the present study, the estimated high flux of manganese, $21 \mu\text{g}/\text{cm}^2 \cdot \text{y}$ from the upper slope region of WCMI (Table 2), points out the importance of WCMI in controlling the overall marine budget of manganese in seawater. In this regard, more rigorous and quantitative information on manganese flux calculation is necessitated by studying concentration gradient of manganese in sediment pore waters.

Lower slope sediments (K-11 and M-12)

The manganese distribution in the two cores, K-11 and M-12, from the lower slope region shows features, which are distinctly different from those observed in the upper slope sediment cores. These two cores have high Mn concentrations (~ 1900 and 10160 ppm on CaCO_3 free basis) occurring in surface sections (Figure 2 b, Table 1 b)^{18,28}. In core M-12 the Mn concentration decreases with depth and attains a value of 900 ppm, for K-11 the Mn concentration shows a shallow depth ($4\text{--}5$ cm) maximum and then decreases to a value of ~ 700 ppm below 10 cm depth. Such a distribution of Mn is typical of the slope sediments where Mn released at depths in the sedimentary column by diagenetic processes is sequestered at the oxic sediment–water interface^{15,33,35}. In contrast to the average value of Mn for upper slope sediments, the median value (Median of Mn concentration has been taken owing to large downcore variations in manganese data, see Figure 2 b) of Mn concentration (on a CaCO_3 free basis) taken over the entire core length for K-11 and M-12, is ~ 1340 and ~ 1270 ppm respectively. These values of Mn are comparable with that of fluvial sediments transported by the Narbada/Tapti rivers¹⁶. These data suggest that there is no net mobilization of Mn, but only its redistribution within the column.

A model calculation has been done to determine manganese reduction rate, which in turn provides carbon oxidation flux for the lower slope sediments in the column itself. For this, out of the two cores, only M-12 could be modelled using the concept of Aller^{5,15}, because required exponential function for manganese distribution could be fitted with this core. In the following, discussion

is made on application of Aller model^{5,15} to elucidate oxidation of C_{org} based on Mn distribution in the core M-12.

In the lower slope sediments, the depth profile of 'excess' Mn concentration can be represented by particle mixing and Mn reduction rate as proposed by Aller⁵. This is represented by

$$\frac{\partial C_s}{\partial t} = D \cdot \frac{\partial^2 C_s}{\partial z^2} - R = 0, \quad (3)$$

where D is the mixing coefficient, C_s is the solid-phase 'excess' Mn concentration and R is the Mn reduction rate. For shorter core length, say about 5 cm, D is generally assumed to be constant^{5,15}. Also, an assumption is made that manganese mobilized by reduction–oxidation process is internal, and there is no leakage of Mn^{2+} to the overlying water. The 'excess' manganese [i.e. total solid-phase Mn minus the average background (background Mn concentration refers to unreactive Mn which remains almost constant after a certain depth from the surface) value in $\mu\text{g}/\text{g}$] in top ($0\text{--}4$ cm) of the core M-12 is converted in units of mass/volume ($\mu\text{M}/\text{cm}^3$) by using *in-situ* sediment density of $0.33 \text{ g}/\text{cm}^3$ and its depth profile is fitted with an exponential function⁴:

$$C_s = C_0 \cdot e^{-\beta \cdot z}, \quad (4)$$

where C_s and C_0 are the solid phase Mn concentration at any depth and at the sediment surface respectively and β is the attenuation coefficient. Attenuation constant

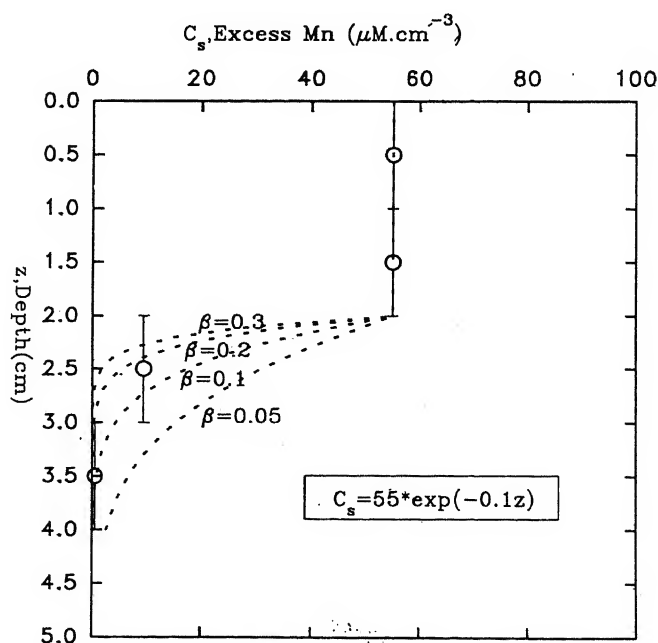


Figure 3. Experimental data for the sediment core M-12 fitted with the exponential function for different values of β (see text for explanation).

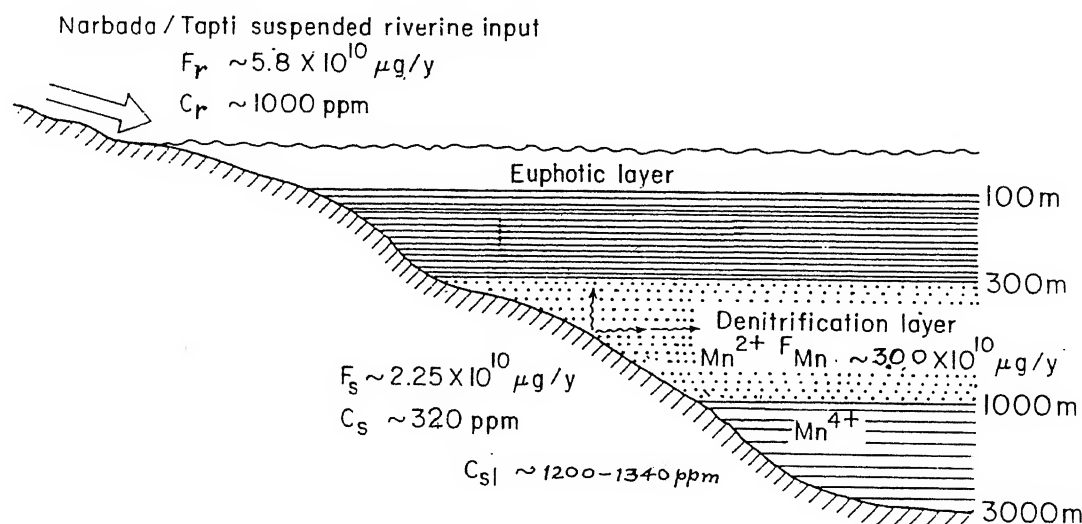
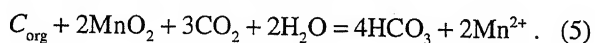


Figure 4. Schematic diagram showing Mn cycling between sediment and seawater along the WCMI region that represents $5^\circ\text{Lat.} \times 3^\circ\text{Long.}$ area. C_r is concentration and F_r the flux of Mn transported by riverine suspended matters. C_s and F_s refer to concentration and flux of Mn mobilized from reducing upper slope sediments to the denitrification layer. C_{sl} is the average concentration of Mn in lower slope sediments. Although dissolved riverine Mn input and atmospheric transport are neglected, a reasonable degree of balance is observed between F_r and $(F_s + F_{Mn})$.

(β) controls decay profile of 'excess' manganese concentration with depth which is dependent on particle mixing and bioturbation. The best fit with experimental data by the exponential function (with varying β) is shown in Figure 3. It is clear from the figure that the most suitable β value for Mn distribution in the core is 0.1 cm^{-1} . The only unknown in eqn (3) is D for determining (R), the Mn reduction rate. Assuming that the exponential depth profile of $^{210}\text{Pb}_{\text{excess}}$ in top 10 cm of M-12 is solely due to particle mixing (assumption seems to be reasonable since bioturbation is important for lower slope sediments owing to prevalence of oxic conditions at the sediment-water interface), the mixing coefficient D (ref. 36) is calculated to be $0.18 \text{ cm}^2/\text{y}$ which is an upper limit for particle mixing in the core M-12. This value of mixing coefficient is within the range ($0.04\text{--}0.4 \text{ cm}^2 \cdot \text{y}^{-1}$) (ref. 37) often cited for deep sea sediments.

Using this value of D and exponential function for 'excess' Mn concentration in eqn (4) which results R , the Mn reduction rate, integral of R over the depth interval for which 'excess' Mn is observed, yields an average Mn reduction flux of $0.33 \mu\text{M}/\text{cm}^2 \cdot \text{y}$. This information on Mn reduction flux is related to C_{org} oxidation flux within the sedimentary column, the details of which are given below.

The manganese reduction in nearshore sediments is represented by the following relation⁹



This shows that two moles of Mn^{2+} are generated during the oxidation of one mole of C_{org} . Therefore, it

is possible to estimate the amount of C_{org} oxidized from the Mn^{2+} released based on this stoichiometry. Such an estimate in the present study would be an upper limit for the amount of organic carbon mineralized because of two reasons: (i) uptake of Mn^{2+} by manganese carbonate precipitation in sediments from pore waters^{13,14} is neglected, and (ii) reduction of Mn oxides by abiotic/inorganic reactions³⁸ is ignored.

Considering manganese reduction flux of $0.33 \mu\text{M}/\text{cm}^2 \cdot \text{y}$ and following eqn (5), the flux of organic carbon oxidized in lower slope sedimentary column is $\sim 0.16 \mu\text{M}/\text{cm}^2 \cdot \text{y}$. Similarly, based on estimate of Mn flux (range, $7\text{--}69 \mu\text{g}/\text{cm}^2 \cdot \text{y} \equiv 0.1\text{--}1.0 \mu\text{M}/\text{cm}^2 \cdot \text{y}$) mobilized from the upper slope, the oxidation rate of C_{org} is calculated to be in the range of 0.05 to $0.5 \mu\text{M}/\text{cm}^2 \cdot \text{y}$. Comparing this flux with the primary production ($\sim 1500 \mu\text{M C}/\text{cm}^2 \cdot \text{y}$) as reported for upper slope region³⁹, it implies that the reduction of Mn contributes to $<1\%$ oxidation of C_{org} fixed by the photosynthetic activity. This indicates that C_{org} oxidation up to a stage of Mn reduction in sediments from the upper slope region may be a lower limit for organic matter combustion. More extensive study of this kind is required over the Arabian Sea region to establish relationship between manganese reduction rate and the carbon oxidation flux at the sediment-water interface. The results obtained from this study have direct relevance to the objectives of the ongoing JGOFS (India) programme.

The manganese cycling between solid and aqueous phases along the WCMI has been shown in Figure 4. A reasonable degree of balance is observed between the flux of manganese transported through Narbada/Tapti riverine sediments ($F_r \sim 5.8 \times 10^{10} \mu\text{g/y}$) and the sum of

fluxes that mobilized into seawater and the net burial rate of Mn ($F_s + F_{Mn} \sim 5.3 \times 10^{10} \mu\text{g/y}$) in the upper slope sediments. F_s , the net burial flux of manganese is calculated by assuming typical concentration of ~ 300 ppm, density of 0.5 g/cm^3 and rate of sediment accumulation 1 mm/y for the upper slope region. Furthermore, an assumption is made that contribution of Mn from its dissolved component through rivers and atmospheric transport is negligible. Figure 4 shows that the median value of Mn over the core length for the lower slope sediments is comparable with that of river suspended particles and average crustal rock composition. Thus, isopycnal transport of soluble manganese from upper slope sediments to oxidizing lower slope sediments, as is expected, is not a significant phenomenon. In the lower slope sediments, therefore, most likely the manganese cycling is internal in nature and its depth profile is controlled by microbial diagenetic processes.

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Conformation of ATPMg(II) bound at the specific site on bovine serum albumin: ^1H -nuclear magnetic resonance study

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Bovine serum albumin (BSA) binds one molecule of adenosine 5'-triphosphate (ATP) at one of the fatty acid-binding sites. Interaction of ATPMg(II) with BSA and its conformation in the bound form has been investigated using ^1H -nuclear magnetic resonance (NMR). Binding of nucleotide with albumin results in downfield shift of all the ligand proton resonances, indicating the involvement of adenine as well as ribose moiety in binding interactions. Measurements of change in chemical shifts of H2, H8 and H1' protons of the ATP in the bound form as a function of ligand concentration, has yielded a dissociation constant of $K_d \sim 2$ mM for ATPMg(II) with albumin. Two dimensional transferred nuclear Overhauser spectroscopy (TRNOESY) was used to determine the inter-proton distances in ATPMg(II) bound to BSA. In order to select a concentration range of the ligand where binding occurs exclusively at the specific site, we have measured the NOE intensities for H1'-H2' protons as a function of ATP concentration. For determining the conformation of bound ATPMg(II) at the specific site, NOE intensities have been measured for several mixing times ($\tau_m = 40$ –200 ms). The resulting NOE buildup curves were analysed using complete relaxation matrix calculations to determine various inter-proton distances. Examination of amino-acid sequence of BSA has led to the identification of a region GVKGLSRS (aminoacyl residues 425–432) which is very similar to the consensus sequence present in several ATP-binding proteins. On the basis of observed similarities in the conformation of bound ATP with that of other ATP-utilizing enzymes and the presence of the nucleotide binding consensus sequence, we propose that the specific ATP binding site is located on Domain-III of BSA.

SERUM albumin is one of the most abundant non-glycosylated proteins in the blood plasma. It contributes about 80% of the colloidal osmotic pressure which provides the driving force to keep fluid within blood vessels^{1,2}. The most unique feature of the albumin is its ability to bind a wide variety of biological materials. This includes divalent cations, a variety of metabolites, hormones, drugs, free fatty acids, etc.^{1,3,4}. Several of these functions depend on the extraordinary ability of albumin

to bind hydrophobic as well as hydrophilic compounds. Recent X-ray studies on human serum albumin complexed with different ligands have shown that the principal ligand binding sites were located in subdomains IIA and IIIA (ref. 5). Several aromatic compounds bind at these two sites. Their general orientation in the binding cavity depends upon the nature of substitutions in the aromatic ring.

In a recent study Bauer *et al.*⁶ used a number of spin labelled derivatives of ATP and studied their binding to bovine serum albumin (BSA) using the electron spin resonance method. It was found that ATP binds at a specific site on BSA with a stoichiometry of 1:1 and a dissociation constant in the range of 50–100 μM . BSA is known to bind about 4–5 moles of fatty acids per mole of protein^{7,8} and it is proposed that nucleotide interacts at one of these amphipathic regions on the surface of the protein⁶. ATP with an aromatic ring (adenine), a hydrophilic sugar moiety (ribose) and a highly charged triphosphoryl tail, appears to be an excellent probe to characterize the specific binding site on the albumin. With this in view, we have studied the interaction of ATPMg(II) with BSA using NMR. The ability of NMR to observe every ligand proton has allowed us to identify the regions of the ligand which are in direct contact with the protein in the protein-ligand complex. Further, the conformation of the bound nucleotide has been determined, using proton transferred nuclear Overhauser effect spectroscopy (TRNOESY)^{9–11} in 2D-NOESY mode¹² and has been compared with those of other nucleotide-binding proteins^{13–23}.

Materials and methods

Materials

Bovine serum albumin was purchased from Armour Pharmaceutical Company. ATP, 4.9 M – MgCl_2 solution and D_2O (99.96 atom% D) were obtained from Sigma. Chelex-100 was supplied by Bio-Rad. Tris(hydroxymethyl)aminomethane- d_{11} (99.0 atom% D) (Tris- d_{11}) was purchased from ISOTEC Inc, USA. All other chemicals were of Analar grade and used without purification.

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Sample preparation

Bovine serum albumin was dissolved in 20 mM sodium phosphate, pH 7.5, and dialysed against the same buffer. The solution was treated with Chelex-100 to remove trace metal ions. H₂O was exchanged with D₂O by dialysing the sample against 20 mM sodium phosphate, pH 7.5, (without any isotopic correction) in a dialysis cell. At least ten changes of buffer were made for solvent exchange. This was lyophilized once and then dissolved in 99.96% D₂O. The concentration of BSA was determined spectrophotometrically with $A_{280} = 0.66 \text{ mg}^{-1} \text{ ml cm}^{-1}$ and molecular weight of 66,000 Daltons²⁴. Nucleotide solution was also passed through Chelex-100 column, lyophilized and dissolved in D₂O. The concentration of ATP was determined using $A_{259} = 15.4 \text{ mM}^{-1} \text{ cm}^{-1}$.

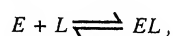
NMR measurements

¹H-NMR measurements were made on a Bruker-AMX500 NMR spectrometer. In all the measurements, sample temperature was maintained at 10°C. Chemical shifts were measured relative to the internal standard, sodium trimethylsilyl propionate-2,2,3,3-d₄ (TSP). 1D-¹H NMR spectra were recorded with 16 K data points, 6024 Hz spectral width, 2 s relaxation delay and 64 transients were averaged for each spectrum. NOESY time domain data were acquired with 256 t_1 increments and 2 K t_2 points in the phase sensitive mode with the use of TPPI²⁵. Sixty-four scans were averaged for each t_1 increment. Measurements were made with mixing times 40, 80, 120, 160 and 200 ms. The carrier frequency was placed at the HDO resonance in all the experiments. The solvent resonance was suppressed by irradiation using the decoupler channel during intervals of relaxation delay, the t_1 period, and the mixing time. The acquisition parameters were, 1.5 s relaxation delay, 0.17 s acquisition time, and 6024 Hz sweep width.

Data were processed on an Aspect-X32 computer with Bruker software. All the data were processed identically with a two-dimensional Fourier transformation along both dimensions with a Gaussian apodization and a zero filling in t_1 to obtain 1 K (F1) × 2 K (F2) real data points. The spectra were phased to pure absorption mode. The volume of the cross peaks was determined by integration. Percentage of NOEs was derived by dividing the observed cross peak volume by the diagonal peak volume of H1' extrapolated to zero mixing time and then multiplying it by 100. In experiments where measurements were made only for a single mixing time, the diagonal intensity of H1' in the same spectrum was used to determine the per cent NOE.

Determination of binding constant

In an equilibrium between a macromolecule E and ligand L ,



the dissociation constant is given by

$$K_d = [E][L]/[EL].$$

For a given value of K_d , E_T and L_T , where E_T and L_T are the total concentrations of the macromolecule and the ligand; the mole fraction of the bound ligand (P_M), i.e. $[EL]/[L_T]$ can be derived easily. Further, if the binding of L to E results in a change in chemical shift of L resonance by $\Delta\omega$, then under conditions of fast exchange it can easily be shown that

$$\Delta\omega = P_M \Delta\omega_M,$$

where $\Delta\omega_M$ is the chemical shift of the fully bound form of the ligand with respect to its position in unbound form²⁶. Thus, the measurement of the change in the chemical shift where a fixed concentration of macromolecule is titrated with varying concentrations of the ligand, can be analysed by two parameter iterative fit to derive the values of K_d and $\Delta\omega_M$.

Relaxation matrix calculations

Simulations of complete NOE buildup curves in a TRNOESY experiment are greatly simplified if the fast exchange condition prevails²⁷, i.e. the 'on' and 'off' rates of the ligand must be faster than the typical cross relaxation rates^{21,28}. In the limits of fast exchange, the intensity of the $i \leftarrow j$ cross peak represents a polarization transfer from spin j to i , for a mixing time τ_m and is given by^{18,20,21}

$$m_{i \leftarrow j}(\tau_m) = (e^{-R\tau_m})_{ij} M_{oj} \quad (1)$$

$$= [1 - R\tau_m + \frac{1}{2} R^2 \tau_m^2 - \frac{1}{6} R^3 \tau_m^3 + \dots]_{ij} M_{oj}, \quad (2)$$

where M_{oj} is the equilibrium value of the j spin magnetization and R is an average relaxation matrix given by

$$R = p_b W^b + p_f W^f, \quad (3)$$

where p_b and p_f are bound and free fractions of ligand each containing n -spins^{27,29,30}, W^b and W^f are the dipolar interaction^{27,31-34} terms between two protons in bound and free form respectively and are given by

$$W_{ij} = W_{ji} = \frac{\gamma^4 \hbar^2 \tau_c}{10 r_{ij}^6} \left[-1 + \frac{6}{1 + 4\omega^2 \tau_c^2} \right] \quad (4)$$

and

$$W_{ii} = \frac{\gamma^4 \hbar^2 \tau_c}{10} \left[1 + \frac{3}{1 + \omega^2 \tau_c^2} \frac{6}{1 + 4\omega^2 \tau_c^2} \right] \sum_{k \neq i} r_{ik}^{-6}, \quad (5)$$

in which γ and ω are the gyromagnetic ratio and Larmor frequency of the protons, r_{ij} is the distance between spins i and j , and τ_c is the isotropic rotational correlation time. Equations (4) and (5) assume that the spin system is in a single conformation characterized by distance r_{ij} , and undergoing isotropic rotational diffusion characterized by the τ_c . Equation (2) shows that, for short mixing times, the build-up of the intensity of a cross peak in a TRNOESY spectrum, given by $m_{i \leftarrow j}(\tau_m)$ vs τ_m , is a polynomial in τ_m , and the initial slope of the build-up which is just the linear term, yields R_{ij} . Since usually $\tau_c^b > \tau_c^f$, p_b/p_f is 0.1–0.15, the last term in equation (3) is negligible so that

$$R_{ij} = P_b W_{ij}^b. \quad (6)$$

From equations (4) and (5) it can easily be shown that the ratio of initial slopes for different spin pairs is related to the corresponding internuclear distances in the bound conformation as

$$(R_{ij}/R_{ik}) \approx (r_{ik}^b/r_{ij}^b)^6. \quad (7)$$

Here $r_{ij}^b = 2.90 \pm 0.2 \text{ \AA}$ is the calibration distance between H1' and H2'. This distance is known to be independent of the nucleotide conformation^{15,35}. The inter-proton distances derived using equation (7) were used as initial distances and a comprehensive analysis of data was done using equations (1) and (2) to obtain an iterative fit between theory and experiment.

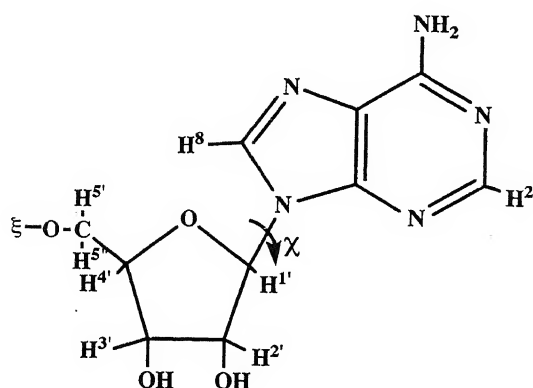


Figure 1. Schematic diagram of adenosine moiety of ATP, indicating the proton numbering system used here.

Energy minimization and molecular modelling

Molecular modelling and restrained energy minimization were performed using Insight II (V2.3.0) in the software package, Discover (V2.9.5) supplied by Biosym Technologies. The software was run on Iris Indigo work station supplied by Silicon graphics computer systems. The calculations were performed on adenosine molecule in vacuum. The inter-proton distances derived after complete relaxation matrix calculations were used as restraints allowing $\pm 10\%$ variation in the distance. The energy was minimized using steepest descent method followed by conjugate gradients method available with the software package.

Results and analysis

Binding of ATPMg(II)

In $^1\text{H-NMR}$ spectrum of ATPMg(II), resonances due to H2, H8, H1' (see Figure 1 for nomenclature of various protons in ATP) are observable at 8.51, 8.22 and 6.14 ppm respectively. Binding of ATPMg(II) to BSA results in downfield shift of all the three resonances. The H2 proton resonance of adenine ring exhibits the largest shift. A sample containing 0.50 mM BSA in 25 mM Tris- $\text{d}_{11}\text{-Cl}$, pH 7.5, and 25 mM MgCl_2 , was titrated with ATP in the concentration range of 0.43–4.73 mM. The observed change in the chemical shift of a proton was measured in Hz, from the corresponding resonance of ATPMg(II) in the absence of BSA. Largest downfield shift was observable at the lowest concentration of ATP. With increasing concentration of ATP, the resonances tend to shift towards the unbound ATP positions. Such behaviour is characteristic of a fast exchange condition where the rate of exchange of a ligand between bound and free environment is faster than that of the difference in chemical shift in two states. The data from this experiment have been analysed (as described above) to determine the dissociation constant for ligand binding (K_d) and the change in chemical shift for various protons in the bound form ($\Delta\omega_M$). Figure 2 presents the observed change in chemical shift measured from the respective free ligand resonance as a function of mole fraction of the bound ligand. The dissociation constant (K_d) derived for ATPMg(II) is $2.00 \pm 0.15 \text{ mM}$. Downfield shift of H2, H8 and H1' resonances in bound form with respect to free form are 191 ± 12 (0.382 ppm), 76 ± 6 (0.158 ppm), and 66 ± 5 (0.132 ppm) Hz respectively. In a simple case of two site exchange, if ω_A and ω_B are the resonance frequencies at the two sites (free and bound respectively) in the absence of exchange and τ_A and τ_B are the corresponding lifetimes at the two sites when exchange occurs, the fast exchange condition³⁶ can be defined by

$|\omega_A + \omega_B| = \Delta\omega_M < \tau_A^{-1}, \tau_B^{-1}$. Since the τ_B is the lifetime of ligand in *EL* complex, τ_B^{-1} represents the rate of dissociation of ligand (k_{off}) from the complex. Thus, it can be shown that under fast exchange condition, $\tau_B^{-1}(k_{\text{off}}) > \Delta\omega_M (2\pi\Delta\nu)$. The largest change in chemical shift of H2-proton in the bound form allows us to set the lower limit for the rate of dissociation of ligand from the complex (k_{off}), that is $k_{\text{off}} \gg 1200 \text{ s}^{-1}$.

Dependence of NOE on ligand concentration

Figure 3 presents a typical NOESY spectrum of BSA.ATPMg(II), where several ligand inter-proton NOEs are observable as off diagonal peaks. To observe the site-specific NOEs arising due to interaction of ligand at the site of interest, appropriate sample conditions must be determined¹⁸. We have measured TRNOESY spectra with a mixing time of 120 ms at several different concentrations of the ligand. In each case the ratio of protein to ligand was maintained at 1:10. Note that protein concentration will also vary along with the ligand. Separate samples were prepared for each ATP concentration to avoid errors due to dilution. Figure 4

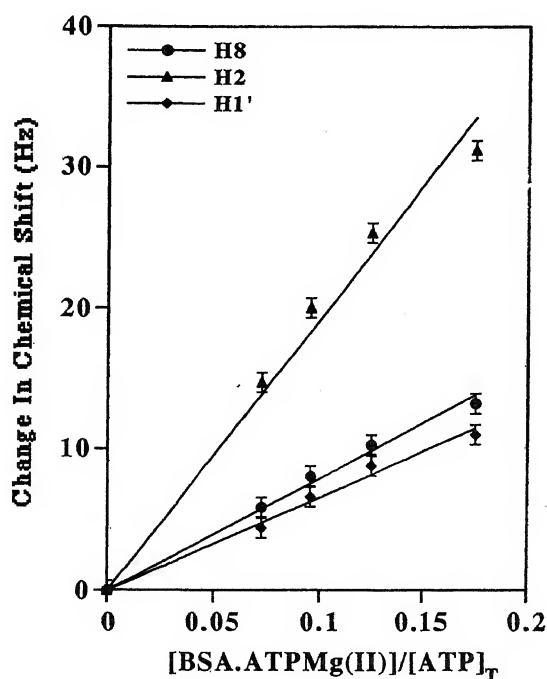


Figure 2. A plot of observed downfield change in chemical shift (Hz) of H2, H8 and H1' protons of ATP measured from the respective free resonances, as a function of bound ATP mole fraction ($[\text{BSA.ATPMg(II)}]/[\text{ATP}]_T$). Sample contained 0.50 mM BSA; 20 mM MgCl_2 ; 25 mM Tris-(d_{11})-HCl pH 7.5 and the ATP concentration was varied from 0.43 to 4.73 mM. The spectra were recorded at 500 MHz, 10°C with a digital resolution of 0.35 Hz. The points are experimental and the solid curves are computed with a $K_d = 2.0 \text{ mM}$ for BSA.ATPMg(II) complex and the difference in chemical shift for bound and free resonances to be 191 ± 12 , 76 ± 6 and $66 \pm 5 \text{ Hz}$ for H2, H8 and H1' protons respectively.

shows a plot of per cent NOE for H'-H2' (distance between these two protons is invariant, irrespective of the nucleotide conformation) as a function of ATP concentration. Initial increase in per cent NOE to ~2% for a ligand concentration of 5 mM is indicative of gradual saturation of specific nucleotide-binding site. Observed increase in per cent NOE at ligand concentrations >5 mM may arise due to weak binding of ATPMg(II) at some adventitious site(s). Similar behaviour has been observed in case of rabbit muscle creatine kinase¹⁸ and yeast pyruvate kinase²⁰. From these data it is clear that for the determination of bound nucleotide conformation, measurements must be performed on a sample with a ligand concentration <5 mM.

Structure-dependent intramolecular NOE measurements

For the determination of conformation of adenosine moiety bound to BSA, measurements were made on a sample containing 4.0 mM ATP. Such sample composition maximizes the binding of ligand at the specific site and restricts interaction at low affinity non-specific sites.

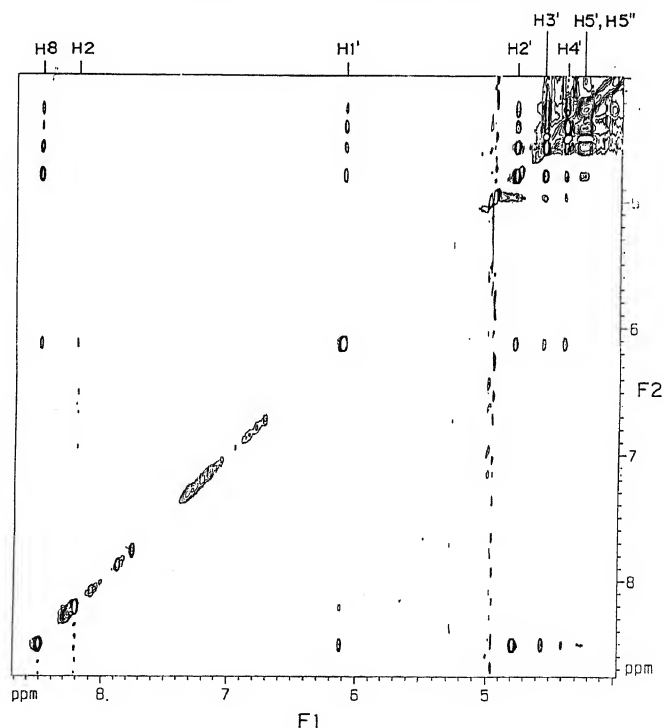


Figure 3. TRNOESY spectrum of BSA.ATPMg(II) complex recorded at 500 MHz and 10°C. The sample contained 0.572 mM BSA; 4.0 mM ATP; 20 mM MgCl_2 in 20 mM sodium phosphate, pH 7.5. The sample volume was 600 μl . NOESY time domain data were collected with $256 \times t_1$ increments and 2 K points during each accumulation in t_2 . The mixing time of 160 ms and a relaxation delay of 2 s were used for the spectrum presented here. Two-dimensional Fourier transformation was performed along both the dimensions with a Gaussian apodization and zero-filling to obtain 1 K (F1) \times 2 K (F2) data set. The spectra were phased to pure absorption phase.

The mole fraction of bound ligand (P_b) was also within the range (~ 0.1 – 0.25) as suggested by Campbell and Sykes^{30,37} on the basis of theoretical considerations. The final sample composition chosen for NOE measurements was 0.572 mM BSA, 4.0 mM ATP and 20 mM MgCl_2 in 20 mM sodium phosphate, pH 7.5. Under such sample condition and for a $K_d = 2$ mM, P_b is ~ 0.1 . The experimentally measured NOE buildup curves are shown in Figure 5, where per cent NOE is plotted as a function of mixing time for different proton pairs. The points represent experimentally measured values, while the solid curves were theoretically simulated as described below.

NOE buildup curves (shown in Figure 5) were analysed to obtain the inter-proton distances. The data analysis was performed in a similar way as was done for creatine kinase and arginine kinase^{18,19}. Initially, the experimental NOEs were fitted with a second order polynomial in τ_m , including (0, 0) point as a part of data. Two distinct resonances are observed for $\text{H5}'$ and $\text{H5}''$ of free ATP. However, in the presence of Mg(II) , these two resonances overlap. In the analysis of data here, these two protons were treated as a single proton and observed cross peak intensities involving $\text{H5}'$ and $\text{H5}''$ were divided by a factor of two before fitting them with the polynomial. Using a calibration distance of 2.9 Å for $\text{H1}'\text{H2}'$ (refs 15, 20) in conjunction with equation (7) and the initial slopes obtained for different proton-pairs, a set of inter-proton distances were determined. NOE data for $\text{H1}'\text{H2}'$ with the calibration distance of 2.9 Å, also yields a value of $P_b\tau_c^b$ [see equations (4) and (6)]. These two parameters were separated by determining the P_b from the known dissociation constant. The set of initial slope distances, P_b and τ_c^b , obtained in this manner were

used as a starter for complete relaxation matrix calculations. The cross peak intensities were calculated for each spin pair as a function of mixing time (τ_m) according to equations (1), (3) and (4). To account for the contribution of free ATPMg(II) [see equation (3)], a τ_c^f of 0.3 ns (ref. 38) and inter-proton distances from an energy minimized structure were used. To obtain a better fit for the data at larger mixing times, a leakage term was added to account for the contributions arising from any external relaxation mechanism (see legend to Figure 5). The buildup curves computed theoretically on the basis of these parameters were then compared with the experimental data. At this stage any of the parameters, i.e. τ_c^b , r_{ij} and leakage terms could be adjusted to obtain the best fit for the experimental data. The bound correlation time, τ_c^b , chosen for the best fit of experimental data was 53 ns. The inter-proton distances determined from the analysis of TRNOE data are given in Table 1 along with the initial slope distances. The solid curves were computed using this set of distances along with other parameters mentioned in the legend of Figure 5. The distances derived from initial slope approximation and relaxation matrix calculations are plotted in Figure 6a. It is interesting to note that initial slope approximation underestimates the inter-proton distances which are > 3.5 Å. However, distance < 3.5 Å shows a good agreement between the two methods.

Bound nucleotide structure

The inter-proton distances derived from analysis of NOE data (see Table 1) were used as restraints in energy minimization calculations using Discover (V2.9.5) in conjunction with Insight II (V2.3.0). During minimization, experimentally obtained distances were used as target distances and a variation of $\pm 10\%$ was allowed. A symmetrical simple harmonic function with a force constant of $50 \text{ kcal mol}^{-1} \text{ \AA}^{-2}$ was used to restrain the inter-atomic distances within the range. Since there were no distances determined in the triphosphate chain region of ATP, calculations were performed only on adenosine molecule. Distance restraints were applied sequentially²¹, starting with H8 and all other protons. In the second step, the distances between $\text{H1}'$ and all other protons were used. In the third and the final step, all the distances were used. Each time, the energy minimization was performed using conjugate gradient method. The initial structure obtained in this manner fitted well with target distances. However, the energy of such structure was quite high compared to the energy of the free adenosine molecule. To further refine the initial structure, all the distances were used but the force constant was reduced to $25 \text{ kcal mol}^{-1} \text{ \AA}^{-2}$. This resulted in a structure with significantly lower energy with minor changes in inter-proton distances. Inter-proton distances between

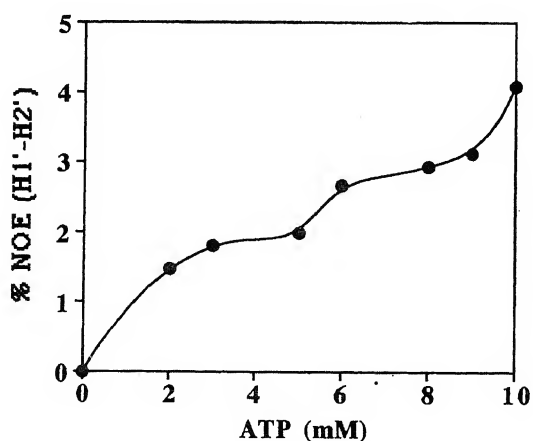


Figure 4. Concentration dependence of per cent NOE for $\text{H1}'\text{H2}'$ proton pair in $\text{BSA}.\text{ATPMg(II)}$ complex for a mixing time of 120 ms. The ATP concentration was varied between 2 and 10 mM. The ATP:BSA ratio was kept at 10:1. The sample contained 2–10 mM ATP; 0.2–1 mM BSA; 20 mM MgCl_2 in 20 mM sodium phosphate, pH 7.5. The experimental points are represented by circles and the solid curves represent smooth interpolation through the experimental points.

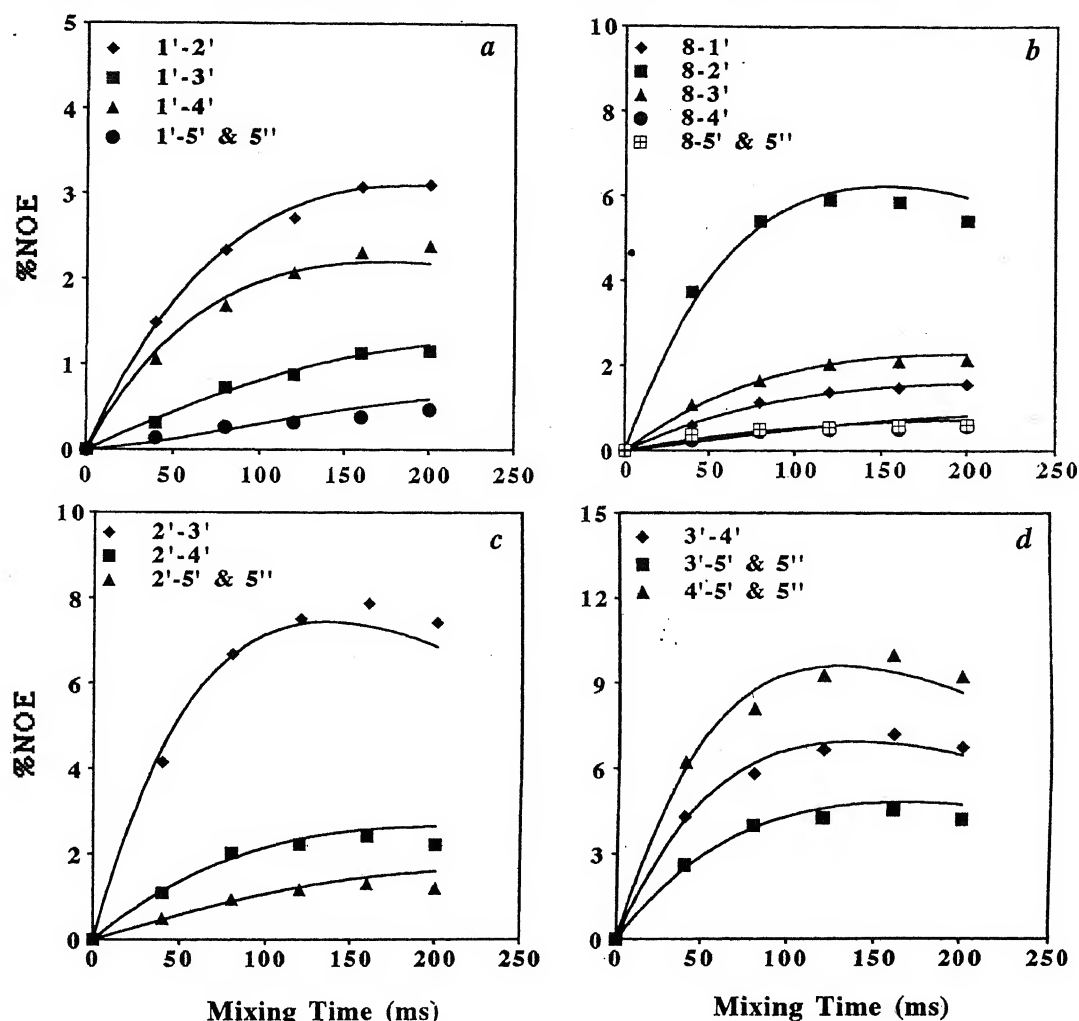


Figure 5. Per cent NOE for different pairs of ATP protons as a function of mixing time for BSA.ATPMg(II) complex. 600 μ l of the sample containing 0.572 mM BSA, 4.0 mM ATP, 20 mM MgCl_2 in 20 mM sodium phosphate, pH 7.5 was used. Spectral acquisition and processing parameters are described in 'Materials and methods'. The points represent experimentally measured cross peak volumes and the solid curves are the simulations based on the relaxation matrix distances given in Table 1. External relaxation rates for H8, H2, H1', H2', H3', H4', H5' and H5'' used in the fitting routine are 4.6, 2.0, 3.33, 3.33, 3.33, 2.86, 4.0 and 4.0 s^{-1} respectively. The rotational correlation time used for the bound ligand is 53 ns.

various pairs of protons in energy minimized structure are listed in Table 1. There are three violations in upper bound (between proton pairs H2'-H4', H2'-H5' and H3'-H5') and two in lower bound distances (between proton pairs H2'-H3' and H1'-H5') (see Figure 6b). Note that in these five violations, three involve H5' whose distance from other protons is difficult to determine accurately. The sum of upper bound violations (SUV) is 1.03 Å and sum of lower bound violations (SLV) is 0.14 Å. Thus the distances in energy minimized structure match reasonably well with those determined by NOEs. The structure of the nucleotide bound to BSA derived above is schematically represented in Figure 7. It has a glycosidic torsion angle $\chi = 48 \pm 5^\circ$, indicating an *anti* configuration of the adenine ring with respect to ribose. The values of various dihedral angles for the sugar ring are given in Table 2. In the notation of

Altona and Sundaralingam³⁹, the phase angle of pseudo-rotation is about 150° corresponding to an unsymmetrical C2'-endo-C1'-exo twist (2T_1) sugar pucker. The amplitude of the sugar pucker, τ is about 16° .

Discussion

Binding of ATP to BSA at a specific site was first reported by Bauer *et al.*⁶. Using appropriate spin labelled nucleotide analogues, these authors determined the binding constant for ATP to be in the range of 50–100 μM . Competitive displacement of bound spin probes by stearic acid suggested that the nucleotide binds at the site where the fatty acids interact. BSA is known to have about five fatty acid sites⁷, with a binding constant $\sim 10^6$ – 10^8 M^{-1} . Differential nature of these sites is evident

Table 1. Inter-proton distances determined for ATPMg(II) bound to bovine serum albumin. The uncertainty in distances is about ± 0.20 Å. Also note that distances of H5' and H5'' from any other proton are not determined separately as both protons resonate at the same frequency. Only in restrained energy minimization, this differentiation is made and separate distances are obtained

Proton pair	Inter-proton distances (Å)		
	Initial slope	Relaxation matrix	Energy minimized
H8-H1'	3.29	3.37	3.66
H8-H2'	2.49	2.50	2.36
H8-H3'	3.05	3.12	3.38
H8-H4'	3.86	4.35	4.11
H8-H5'	3.73	3.90	3.70
H8-H5''	3.73	3.90	4.12
H1'-H2'	2.90	2.90	2.93
H1'-H3'	3.59	3.80	4.00
H1'-H4'	3.06	3.00	3.23
H1'-H5'	4.29	5.50	4.86
H1'-H5''	4.29	5.50	5.19
H2'-H3'	2.42	2.37	2.08
H2'-H4'	2.96	3.05	3.58
H2'-H5'	3.36	3.66	4.39
H2'-H5''	3.36	3.66	4.05
H3'-H4'	2.46	2.40	2.67
H3'-H5'	2.66	2.65	3.36
H3'-H5''	2.66	2.65	2.35
H4'-H5'	2.32	2.25	2.49
H4'-H5''	2.32	2.25	2.51

from the fact that ethanol binds only at three of these sites. Ethanol binding fatty acid sites appear to be the same where 1-anilino-8-naphthalenesulfonate (1,8-ANS) also interacts^{24,40,41}. Further differences in binding determinants at these fatty acid sites are evident from the fact that ATP binds at only one of these sites. This region on the surface of BSA is likely to be amphipathic, i.e. aminoacyl residues present at this site are likely to be apolar and cationic. In the experiments presented here, we have determined the dissociation constant for binding of ATPMg(II) with BSA which is about an order of magnitude larger than that of free ATP (ref. 6). At pH > 7, free ATP has four negative charges whereas ATPMg(II) complex has only two. Adenosine 5'-monophosphate (AMP), which has two negative charges (resembles ATPMg(II) in this respect), also binds weakly with a dissociation constant in the millimolar range⁶. Binding affinity of a ligand to fatty acid binding region of BSA seems to be critically dependent on the number of negative charges on the ligand molecule. The observation of significant changes in the chemical shifts (see Figure 2) of the adenine and the ribose ring protons, indicate that these two rings along with triphosphate chain are involved in direct interaction with the BSA. Although binding of nucleotide to BSA has no known physiological function, interaction of all three regions of ATP with protein suggests that binding domain may resemble other ATP utilizing proteins. We examined the amino-acid sequence of BSA for the presence of a consensus sequence found in several ATP-binding pro-

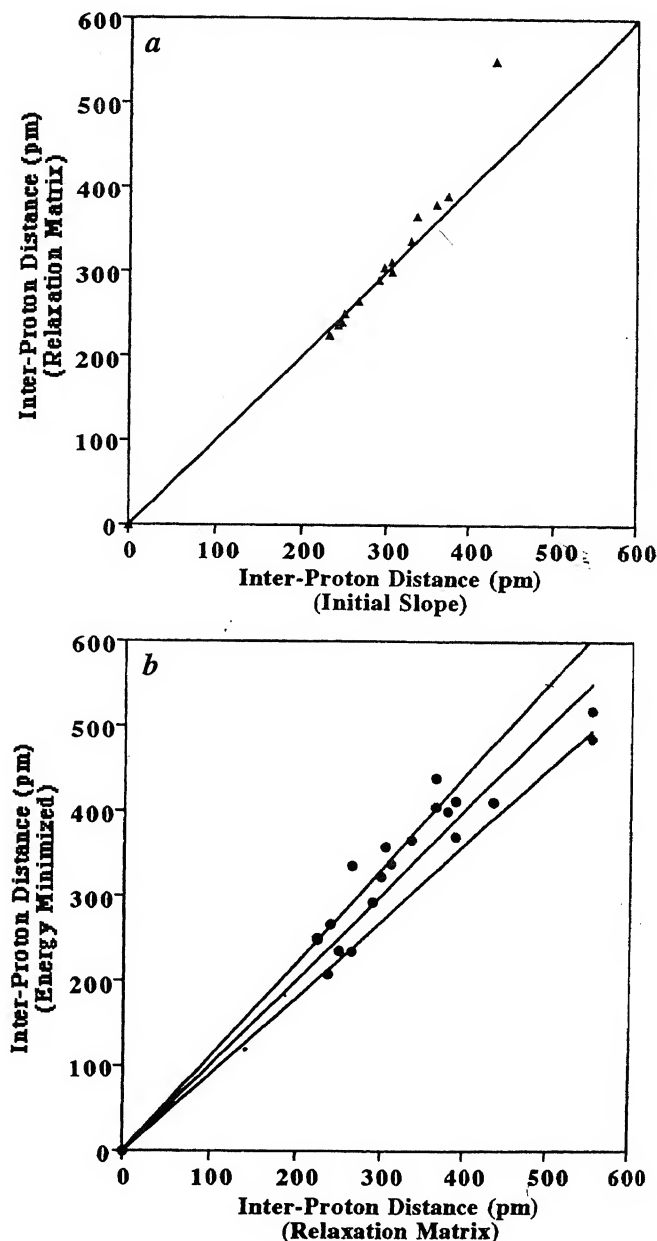


Figure 6. *a*, Comparison of inter-proton distances determined by initial slope approximation and complete relaxation matrix calculations. Fourteen different inter-proton distances determined for ATP in BSA.ATPMg(II) complex are presented. *b*, Comparison of relaxation matrix distances with the model distances derived from the final energy minimized structure of the bound nucleotide.

teins⁴². Aminoacyl residues 425-432, which have a sequence GVKGLSRS, is very similar to ATP-binding consensus sequence. This sequence is part of Domain-III which is known to have one high affinity fatty acid-binding site⁴³. It is quite likely that the specific ATP-binding site is located in Domain-III of the BSA.

In the titration experiment where chemical shifts of ATP protons were measured, we observe that at low

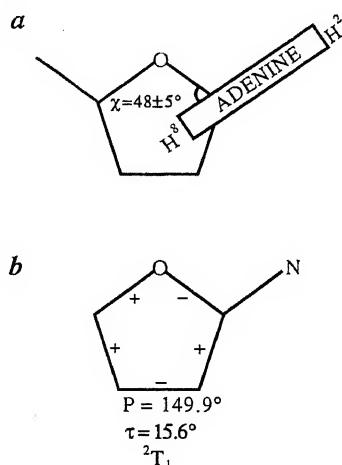


Figure 7. Schematic representation of ATPMg(II) structure bound at the specific site on the BSA (a) the glycosidic torsion angle (χ) and (b) the sign of various torsion angles defining the ribose conformation as well as the phase angle of pseudorotation (p) is mentioned.

Table 2. Different torsion angles and phase angle of pseudorotation (p) for the ribose when bound at the specific site on bovine serum albumin*

Torsion	Angle (degree)
χ (O4'-C1'-N9-C8)	48.0
ν_0 (C4'-O4'-C1'-C2')	-11.6
ν_1 (O4'-C1'-C2'-C3')	15.9
ν_2 (C1'-C2'-C3'-C4')	-13.5
ν_3 (C2'-C3'-C4'-O4')	6.6
ν_4 (C3'-C4'-O4'-C1')	3.3
γ (O5'-C5'-C4'-C3')	68.0
$p = \tan^{-1} \frac{(\nu_4 + \nu_1) - (\nu_3 + \nu_0)}{2\nu_2(\sin 36^\circ + \sin 72^\circ)}$	149.9**
$\tau = \frac{\nu_2}{\cos p}$	15.6

*The definition used for various torsion angles is the same as that described by Sanger⁴⁶.

**Since ν_2 is negative, 180° was added to the p value computed³⁹.

ligand concentration all proton resonances are shifted downfield to different extents. With increasing ligand concentration, the resonances shift progressively, approaching the chemical shift of free ligand at high ligand concentrations. Such behaviour is characteristic of a ligand resonance being in fast exchange²⁸. The largest chemical shift change observed for H2 proton of adenine ring allows us to determine the upper limit for the inverse of life time of BSA. ATPMg(II) complex, i.e. $k_{\text{off}} \gg 1200 \text{ s}^{-1}$. Decrease in binding constant with decreasing negative charges on triphosphoryl chain and observation of downfield shift of proton resonances of adenine and ribose ring suggest that all three regions of nucleotide are involved in binding interactions with the BSA.

The measurement of TRNOE between the H1'-H2' proton pair of ATPMg(II) as a function of ligand concentration (Figure 4) shows that at the higher concentration of ligand, large contributions to observed NOE may arise due to binding at adventitious site(s). In this respect BSA behaves similar to creatine kinase¹⁸ and pyruvate kinase²⁰. Experimental evaluation of the extent of non-specific binding is essential for selection of proper sample composition where site-specific TRNOE measurements could be made. Theoretical analysis of TRNOE data collected under appropriate sample conditions (to maximize specific site binding) implicitly involve the following assumptions. Firstly, the fast exchange conditions prevail, i.e. the inverse of the life time of the ligand in the macromolecular complex should be larger than the typical proton-proton cross relaxation rates. Secondly, it is assumed that all proton pairs have a single effective rotational correlation time (τ_c^b). It is, therefore quite instructive to estimate relaxation rates and compare with the 'off' rate for ATPMg(II) from the protein complex. The 'off' rate for bound ATPMg(II) obtained for BSA. ATPMg(II) complex from chemical shift measurements is $\gg 1200 \text{ s}^{-1}$. Bovine serum albumin, having a molecular weight of 66000 Daltons, may have a rotational correlation time as large as 60–70 ns. The cross relaxation rate between the two protons is given by $\gamma^4 \hbar^2 \tau_c / 10 r^6$ [see equation (4)]. For an inter-proton distance of 1.90 Å and $\tau_c = 70 \text{ ns}$, the cross-relaxation rate is 85 s^{-1} . The cross-relaxation rate computed here is much less than the 'off rate' (k_{off}) of the ligand from the protein complex. Thus, our analysis of TRNOE data using the formulation evolved for fast exchange condition is valid. Further, use of a single rotational correlation time, $\tau_c^b = 53 \text{ ns}$ for all the proton pairs is justified by the fact that the binding of nucleotide with protein involves all three regions of ATP. This will rule out any differential segmental motion for the ligand. The correlation time, $\tau_c^b = 53 \text{ ns}$ which gives the best fit for the experimental data, is in close agreement with the value computed on the basis of Stokes equation (~ 60 – 70 ns). In most of the ATP utilizing enzymes, τ_c^b obtained from the analysis of TRNOE data are much smaller (by a factor of 2–10) compared to Stokes equation estimates^{18–21,44,45} and this has been attributed to unaccounted spin diffusion in bound complexes.

The final structure determined for ATPMg(II) bound to BSA has a glycosidic torsion angle $\chi = 48 \pm 5^\circ$. This value is in general agreement with that obtained for several ATP-utilizing enzymes, viz. creatine kinase ($\chi = 51 \pm 5^\circ$)¹⁸, arginine kinase ($\chi = 50 \pm 5^\circ$)¹⁹, pyruvate kinase (active site $\chi = 44 \pm 5^\circ$, ancillary site $\chi = 46 \pm 5^\circ$)²⁰, phosphoribosyl pyrophosphate synthetase ($\chi = 50 \pm 5^\circ$)²¹, Mut T ($\chi = 53 \pm 9^\circ$)²³ (for AMPCPP). The sugar pucker, which is described by the phase angle of pseudorotation, $p = 149.9^\circ$, determined here is unsymmetrical C2'-endo

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C1'-exo twist (2T_1). The value of p differs greatly for different enzymes, e.g. 42.4° for pyruvate kinase (active site), 70.5° for creatine kinase, 114.9° for phosphoribosyl pyrophosphate synthetase, 130.8° for arginine kinase. Although the binding of ATPMg(II) to BSA has no known physiological function, it is interesting to note that the bound nucleotide has a conformation very similar to that at the active site(s) of several ATP-utilizing enzymes.

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Patterns of large multilamellar vesicular clusters

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Digitized images of dimyristoyl phosphatidylcholine (DMPC) vesicular clusters, obtained from ordinary bright field microscopy were studied. The clusters followed certain scaling behaviour and could be characterized by the mass fractal dimension D and the porosity value P . Structural perturbations caused by the presence of the cytoskeletal protein, spectrin and hydrodynamic perturbations caused by dilution resulted in change in the above mentioned parameters. The results suggest that the collective behaviour of phospholipid vesicles can serve as an important tool to detect minor alterations in the microenvironment.

CLUSTER formation is a phenomenon which has drawn substantial interest from a wide variety of disciplines¹⁻⁷. Clusters can either be spanned (finite) or unspanned depending on whether the aggregate is bound or unbound within a given spatial domain. Contrary to the notion that the clustered assemblies are non-classifiable due to their random formation, it is now believed that minor changes in local interactions or initial conditions may lead to systematic changes in the geometric properties of clusters. Phospholipid vesicles, liposomes, have been widely used as a model system for the study of problems associated with the bilayer membranes, but clusters of spontaneously formed phospholipid vesicles are yet to be classified. We have studied such vesicle clusters under a brightfield microscope by image processing and subsequent analysis of such images to obtain the characteristic parameters for the clusters. In this report we examine the effect of dilution and structural perturbations caused by a cytoskeletal protein, spectrin, known to provide morphological integrity to the erythrocyte membranes.

Dried films of dimyristoyl phosphatidylcholine (DMPC) (Sigma) were hydrated in a buffer containing 5 mM phosphate, 20 mM KCl, 1 mM EDTA, 0.2 mM DTT, pH 8.0, with and without spectrin (200 µg/ml) to form multilamellar DMPC (4 mM) vesicles. White ghosts from goat blood were prepared by hypotonic lysis in 5 mM phosphate, 1 mM EDTA containing 20 µg/ml of PMSF at pH 8.0, following the procedure of Dodge and co-workers⁸. Spectrin was removed from the ghosts in a buffer containing 0.2 mM sodium phosphate, 0.1 mM EDTA, 0.2 mM DTT, 20 µg/ml of PMSF, pH 8.0

at 37°C. Spectrin dimers were purified after 30% ammonium sulphate precipitation by following published protocols⁹.

The DMPC vesicles, after an hour of incubation at room temperature, were spread on a glass slide, and were visualized through the bright field of an ordinary microscope (Model Diastar, Reichert Scientific Instrument, USA). At least four different fields of view were visually scanned to test the gross uniformity of the resultant clusters. The computer-grabbed images were imported into Windows environment (Microsoft Corporation, USA) using MATLAB under Windows (MathWorks Inc., USA). Figures 1 *a* and *b* show the digitized grey images of the DMPC vesicles in absence and presence of spectrin.

Image processing was performed by Image Processing Toolbox procured from MathWorks. In most cases, however, routines specific for the problem, were developed. For processing the indexed images, the first step was thresholding. The optimal choice for threshold grey value was made following the reported method with minor modifications¹⁰⁻¹², where the histogram of grey value of each pixel was taken with 50 number of bins. Then, the overall extraction was carried out with the grey value corresponding to the bin which is 4th in position regarding frequency.

Once the thresholded image is obtained, one may obtain two classes of information. Firstly, one might ignore the porosity space and observe the cluster contour. Secondly, one might be interested in following up how the image pattern changes by rescaling (i.e. by sampling the image points in frame, with an altered grid distribution). Thus the original grey level image in Figure 1 *a* is subjected to thresholding in Figure 2 *a*, which contains the binary information about the porous region as well as the cluster contour. Figure 2 *b* represents the clusters without any indication to the porous region. Rescaling has been illustrated in Figure 2 *c*, which shows how scaling alters the thresholded image (Figure 2 *a*).

To measure the cluster area including pores (A) from the binary image (i.e. to generate Figure 2 *b* from Figure 2 *a*, the image was first fixed in a rectangular frame). To include the porous region in the calculation of area, the following overlay method was followed. Eight separate images were generated by unit pixel translation parallel to positive and negative directions of abscissa keeping the ordinate fixed, parallel to positive and negative directions of ordinate keeping the abscissa fixed and parallel to the four diagonal of the image frame respectively. The translated image points were layered on top of one another and the union of all such points was called the image O' (data for Figure 2 *b*). To obtain rescaled image (Figure 2 *c*), the image co-ordinates were subjected to suitable scaling transformations.

To get the mass fractal dimensions of the clusters we

*For correspondence

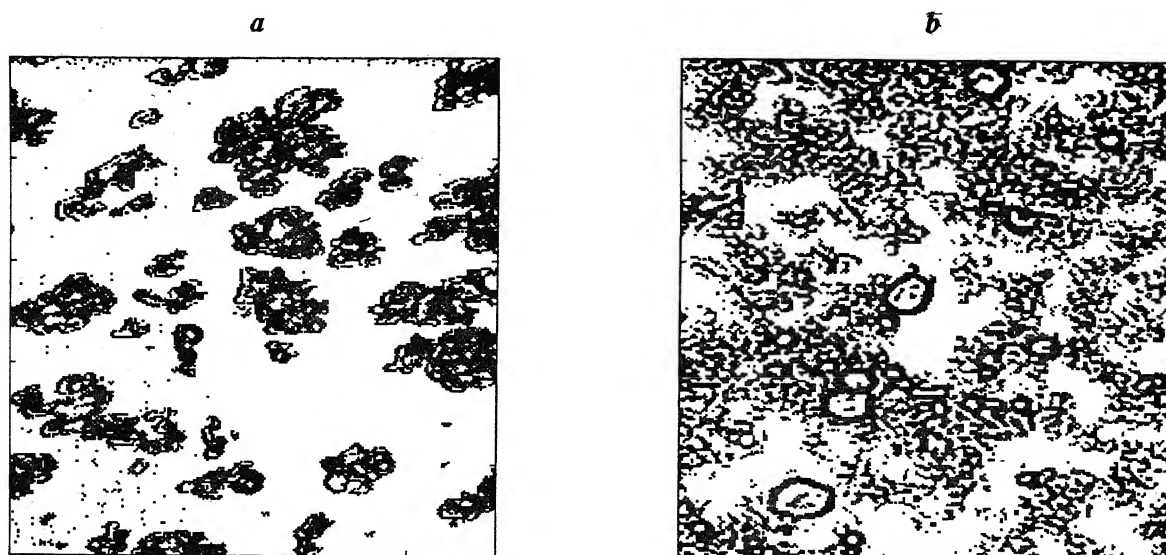


Figure 1 *a, b*. Clusters of DMPC vesicles in (*a*) absence and (*b*) presence of spectrin.

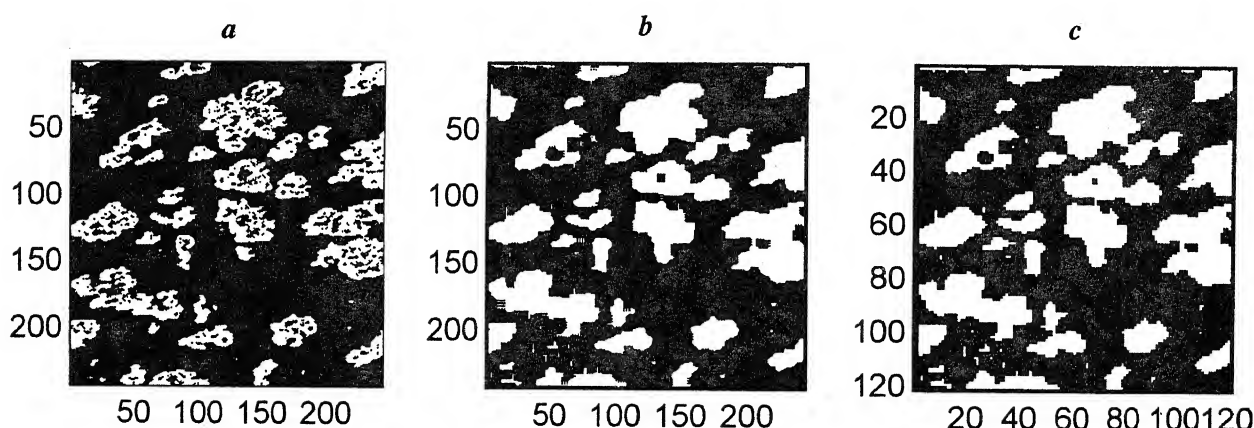


Figure 2 *a-c*. Different stages of processed images (*a*) binary image conversion after thresholding, (*b*) image showing the area within the total cluster contour and (*c*) rescaled image with length scale elongated two-fold (10 pixels = 13.9 μm).

resized all the input thresholded binary images. For this, the input image was scanned blockwise (size of each block was assumed as $a \times a$) each time checking the total grey value, G of each block with the following rule:

if,

$$G(i, j) > 0 \text{ then } R(i, j) = 1; \text{ else } R(i, j) = 0,$$

endif,

where the scale length was represented by a , the size of the input image matrix was m and the size of the output image, R (Figure 2 *c*) was, $L = m/a$. The cluster mass, M (number of white pixels, or occupied sites) of the resized images was measured at different lattice sizes, L ($L = 4, 6, 8, 10, 12, 16, 20, 24, 28$ and 32

pixels). The exponent, D , could be obtained from the equation given by¹³:

$$M = L^D, \quad (1)$$

where the slope of the linear fit of $\ln(M)$ plotted against $\ln(L)$ gives the measure of D , shown in Figure 3.

To evaluate the porosity of the clusters from their two-dimensional view, the expression of the porosity in the three-dimensional space^{14,15} might be modified as

$$P = 1 - A_i/A, \quad (2)$$

where A is the overall cluster area and A_i is the area excluding the pore space.

DMPC films upon hydration with the aqueous buffer

formed large multilamellar vesicles, which upon incubation at 25°C formed stable clusters. When spread on the microscopic slides, spanned clusters were observed without any relative movement between the cluster components. The clustered patterns remained visually similar even when the microscopic slide was physically shifted in the x - y plane. We have obtained a good linear fit in the plots of $\ln(L)$ and $\ln(M)$ (Figure 3), both in absence and presence of spectrin. In the initial stock solution the D value was notably higher in presence of spectrin than that in the absence. After two-fold dilution, a decrease in D was observed in presence of spectrin (200 $\mu\text{g/ml}$), while the D remained unchanged in its absence (Table 1). However, a gradual increase in D , at 2-fold dilution, was evident with the increasing concentrations of spectrin (Figure 3 *b*). Data for evaluation of porosity P , were collected from the threshold-image using eq. (2). For stock solutions we found the mean value of P for clusters of DMPC vesicles to be much lower in the absence of spectrin than that in the presence of spectrin. After two-fold dilution the mean value of P significantly increased for the clusters without spectrin. But on contrary, after 2-fold dilution P remained almost unchanged in the presence of spectrin.

As we see from Table 1, the mean mass fractal dimension, D for the stock DMPC vesicular suspension was significantly lowered (≈ 1.662) in the absence of spectrin than in presence of spectrin (≈ 2.0). The magnitude of D implied that for the DMPC vesicular clusters including the cytoskeletal protein, spectrin, the cluster formation is above the limit of percolation threshold. The cluster formation is below the limit of percolation

threshold when spectrin was not included in the phospholipid vesicles¹³. A gradual increase of D was observed with the increase in spectrin concentration (100–400 $\mu\text{g/ml}$), implying retention of stability in presence of spectrin (Figure 3). Furthermore, for stable clusters one would expect conservation of the geometric properties even in presence of hydrodynamic perturbation. In presence of spectrin the porosity coefficient P , remains relatively insensitive to dilution. This provides an additional support to the earlier conjecture.

Spectrin is the cytoskeletal protein known to provide morphological integrity to the erythrocyte membranes¹⁶. Earlier studies have shown altered vesicular geometry in presence of structural proteins^{17,18}. Most of such studies were confined to the analysis of individual liposomes. Spectrin is known for its membrane adhesive properties and results presented here do support that spectrin confers more stability to the overall cluster characteristics formed by multilamellar DMPC vesicles. The simple methodology presented here might be useful in characterizing clusters from their geometry, despite ambiguity and variety in the microscopic appearances

Table 1. Mean and standard deviation of fractal index and porosity in absence (–) and presence (+) of spectrin

Fractal index (D)		Porosity (P)	
– Spectrin	+ Spectrin	– Spectrin	+ Spectrin
1.66 ± 0.02 (1.69 ± 0.01)	2.02 ± 0.03 (1.75 ± 0.01)	0.16 ± 0.02 (0.45 ± 0.04)	0.44 ± 0.01 (0.39 ± 0.005)

Values in parenthesis correspond to the same after two-fold dilution.

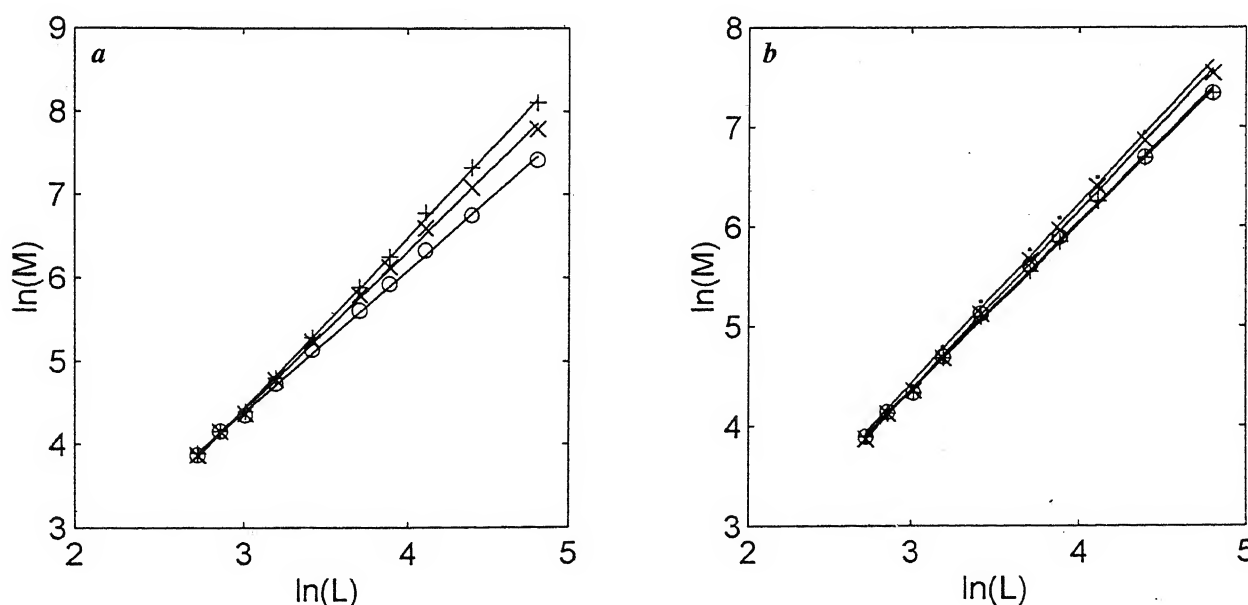


Figure 3 a, b. Plot of $\ln(M)$ against $\ln(L)$. Stock vesicular suspension and two-fold dilution of the same are represented by *a* and *b* respectively. The symbols 'O', '+', 'x' and '*' represent spectrin concentration of 0, 100, 200 and 400 μg per ml respectively.

of their individual vesicular forms. The property of clusters responding to the minute perturbing changes in the individual constituting members, might be, in our view, an important tool in the study of cellular or vesicular assembly processes.

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The plant Banjauri (*Vicoa indica*) exhibits antifertility activity in adult female bonnet monkeys (*Macaca radiata*)

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The antifertility activity of the plant *Vicoa indica* was tested in proven fertile bonnet monkeys. The dry powder of the whole plant was fed to the cycling monkeys on day 1 to 14 of menstrual cycle or day 9 to 14 of cycle or on day 2 to 5 after delivery and the fertility was evaluated in the following cycle in cycle fed monkey or after weaning the young one in the post-partum fed monkeys. Results indicated that while feeding in the post-partum monkeys did not confer any protection against pregnancy feeding during day 1 to 14 of cycle, protected from pregnancy. The monkeys did not become pregnant even after exposure to the proven fertile male monkeys for 13 ovulatory cycles while all the vehicle fed monkeys became pregnant within 3 cycles.

BANJAURI is the colloquial name for the plant *Vicoa indica* which belongs to the family Compositae. It is a

small plant of about 1-2 feet height with slender stem, long leaves and small yellow flowers. The plant grows wild in the months of July/August and fully mature plants with flowers are seen during September/October. It is reported¹ that the Adivasi tribes in Bihar use Banjauri as a contraceptive. According to the practice followed by Adivasis, the freshly collected plant is sun-dried and the whole plant equivalent is powdered along with seven pepper seeds and consumed each day as a suspension in water by woman on the second to fifth day after delivery of the child or from day 1 to 14 of three consecutive menstrual cycles. Such treatment reportedly induces permanent sterility. In view of the nonavailability of scientifically documented clinical study on the use of Banjauri, we have attempted to verify the activity profile of this plant product by determining the effect of feeding dried Banjauri powder both during post-partum period and early phase of the menstrual cycle on fertility of proven fertile adult female bonnet monkeys.

The procedures adopted for care and maintenance of bonnet monkeys have been reported in detail earlier². The serum levels of estradiol-17 β , progesterone and chorionic gonadotropin were determined according to methods described earlier³.

Dried plant material was powdered and passed through a metal sieve (500 μ mesh) to remove coarse fibrous material. The finely powdered material was mixed with groundnut seeds and brown sugar in a ratio of (1:4:8) and thoroughly ground in a mortar and the resulting paste was fed to the monkeys. Care was taken

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to see that the entire material was consumed by the animal and those animals where uncertainty existed regarding intake were discarded from the study. In a separate study, a suspension of Banjauri plant powder in water was deposited in the stomach of female monkeys using a Riles tube.

The first set of experiments were carried out to determine whether the extract is toxic to the monkeys. To a set of monkeys, a total dose of 26 g/monkey (2 g of powder/day) was fed over a period of 13 days. A control set of 3 monkeys received only brown sugar and groundnut powder. The initiation of the feeding of the plant powder to each monkey was done on different days of menstrual cycle as the study was primarily aimed to examine whether the material has any toxic effects on the monkeys. Blood samples were collected before and after the feeding of the powder and analysed for haematological parameters. After feeding the animals were sacrificed and brain, liver, kidney, gonads, muscle and other tissues were collected for histological examination. The blood samples were subjected to haematological, biochemical and endocrinological studies.

Two regimens were employed to ascertain the anti-fertility effect of Banjauri. The first consisted of feeding Banjauri on the 2nd, 3rd, 4th and 5th day following delivery of the young (Group I). In the second, Banjauri

was fed daily from day 1 to 14 of a menstrual cycle (Group II). The post-partum monkey study consisted of two subgroups. While group IA received a total dose of 6 g (1.5 g/day) for 4 days starting from 2nd to 5th day post-partum group IB monkeys were fed a total dose of 60 g (5 g/day given in two doses, one in the morning and one in the evening) over a period of 12 days from day 2 to 14 after delivery. The cycling monkey study was again made up of 2 subgroups. While monkeys in group IIA were given 1.0 g/day for 14 days from day one of the menstrual cycle, those in group IIB were given 2.0 g/day for 14 days from day one of menstrual cycle. A third group (IIC) was given a total dose of 50 g (5 g/dose) from day 1 to day 10 of menstrual cycle.

In the case of monkeys which were fed Banjauri during post-partum period, their fertility was tested following weaning of babies (done six months after delivery) and return to cyclicity (approximately 9–12 months after parturition). In the case of monkeys fed Banjauri during menstrual cycle, the animals were tested for fertility from second cycle onwards.

Female monkeys which had received the drug were placed with proven fertile breeder males (1 : 1) between days 9 and 14 of a menstrual cycle. Those monkeys which did not become pregnant during the first cycle

Table 1. Effect of feeding vehicle (for *Vicoa indica*) for 12 days to bonnet monkeys (*Macaca radiata*) on haematological parameters

	Pre-treatment (n = 3)	Post-treatment (n = 3)	Pre-treatment (n = 4)	Post-treatment (n = 4)
Haemoglobin (g%)	10.6 ± 0.3	11.4 ± 0.4	10.5 ± 0.5	11.25 ± 0.6
PCV (%)	36.0 ± 1.7	33.0 ± 1.5	35.0 ± 2.3	32.8 ± 2.5
TLC (10/cmm)	11.8 ± 1.7	9.4 ± 0.54	10.8 ± 2.3	9.5 ± 1.3
DLC:				
Polymorphs (%)	28.3 ± 3.2	37.0 ± 5.7	39.8 ± 6.0	40.5 ± 8.1
Lymphocytes (%)	64.3 ± 4.3	58.3 ± 4.4	54.5 ± 6.3	54.8 ± 8.4
Monocytes (%)	0	0	0	0
Eosinophils (%)	6.7 ± 2.4	4.7 ± 1.5	0.58 ± 0.1	0.5 ± 0.1
Basophils (%)	0	0	0	0
Platelets (10/cmm)	3.7 ± 0.4	4.2 ± 0.6	3.9 ± 0.3	4.2 ± 0.2
Reticulocytes (%)	0.97 ± 0.1	2.3 ± 0.2	0.7 ± 0.1	2.5 ± 0.3

Values are mean ± SE.

Table 2. Effect of feeding vehicle (brown sugar and groundnuts) for 12 days to bonnet monkeys (*Macaca radiata*) on biochemical parameters

	Pre-treatment (n = 3)	Post-treatment (n = 3)	Pre-treatment (n = 4)	Post-treatment (n = 4)
Total proteins (g%)	7.5 ± 0.2	7.5 ± 0.26	7.85 ± 0.17	7.8 ± 0.3
Albumin (g%)	3.8 ± 0.2	4.3 ± 0.2	3.75 ± 0.08	4.1 ± 0.15
Cholesterol (mg%)	164.3 ± 12.8	160.3 ± 12.8	167.5 ± 5.4	171.8 ± 11.5
SGOT (U/l)	19.3 ± 2.3	24.3 ± 3.7	28.8 ± 6.0	28.8 ± 6.3
Alk. phos. (U/l)	47.7 ± 8.4	32.5 ± 5.7	43.3 ± 3.6	33.5 ± 5.7
Bilirubin (mg%)	0.53 ± 0.09	0.64 ± 0.02	0.48 ± 0.04	0.4 ± 0.8
BUN (mg%)	21.8 ± 2.1	22.2 ± 2.8	26.0 ± 4.8	28.5 ± 0.6
Glucose (mg%)	91.7 ± 6.0	73.3 ± 4.7	84.3 ± 9.9	92.6 ± 10.9

Values are mean ± SE.

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exposure were exposed to fertile males during subsequent cycles.

The ovulatory nature of the cycle and establishment of pregnancy were determined by assaying serum for estradiol 17- β and progesterone and CG by methods standardized earlier in this laboratory³.

Feeding trials revealed that the groundnut powder and brown sugar are very good additives as they completely masked the leafy odour. However, attempts to feed beyond 5 g/day were not successful as the monkeys wasted considerable quantities of the material fed.

Tables 1 and 2 show that there are no gross changes in the haematological, biochemical and histological parameters and as such the plant powder at the dose tried was considered to be non-toxic. Though a detailed study was not carried out even in the monkeys fed 5 g

of Banjauri per day for 10 days no deleterious effects were observed.

Table 3 shows that feeding of 50 g of Banjauri had no significant effect on the serum estradiol on day 9 and 11 and progesterone on day 18, suggesting that Banjauri had no gross effect on the menstrual cyclicity. However, it should be noted that decrease in serum progesterone levels (less than 2 ng/ml Banjauri fed in monkeys compared to >2 ng/ml in control monkeys) did not result in any alteration in the length of the menstrual cycle.

Table 4 also shows that all the control monkeys fed vehicle become pregnant within 2.2 cycle exposures to proven fertile male monkeys, an observation which is in agreement with the earlier results⁴.

Table 5 shows that the monkeys fed either 6 g or

Table 3. Serum estradiol from day 1 to 10 of menstrual cycle and progesterone levels in vehicle fed ($n=3$) and *Vicoa indica* (50 g/day $n=4$) fed monkeys

Group	Duration of cycle (days)	Serum estradiol (pg/ml) on		Serum progesterone (ng/ml)
		Day 9	Day 11	Day 18
Control	29 \pm 3	136 \pm 17	236 \pm 21	1.52 \pm 0.2
<i>Vicoa indica</i>	27 \pm 5	242 \pm 50	178 \pm 18	1.60 \pm 0.5

Mean \pm SE.

Table 4. Effect of feeding dried Banjauri plant powder during post parturition period on fertility in the female bonnet monkey

Group	n	Total dose (g)	No. of ovulatory cycles/ total no. of cycles exposed	Mean no. of cycles needed to become pregnant	Parameter
Control	5	6 (vehicle)*	11/16	2.2	All delivered live young
IA	6	6 (Banjauri)	18/30	3.0	All delivered live young
IB	5	50 (Banjauri)	6/9	1.2	All delivered live young

*A mixture of groundnut powder and brown sugar was used as vehicle. The total dose was given orally in 4 equal portion on days 2, 3, 4 and 5 of lactation.

Each animal was exposed to a proven fertile male during day 9–14 of each cycle. The cycle was considered ovulatory if serum estrogen on day 8/9 was at least 200 pg/ml (range 200–960 pg/ml) and serum progesterone on day 18/19 was at least 2 ng/ml (range 2–9.6 ng/ml).

Table 5. Effect of feeding dried Banjauri plant powder during menstrual cycle in proven fertile female bonnet monkeys on their ability to conceive during subsequent exposure to fertile males

Group	n	Dose (g/day)*	No. of ovulatory cycles/ total no. of cycles exposed	Mean no. of cycles needed to become pregnant	Parameter
IIA	4	1.0	14/16	3.5	All delivered live young
IIB	6	2.0	82/126	–	None became pregnant even after 13.7 ovulatory cycle exposures
IIC	3	5.0	17/	–	One became pregnant after 6 cycles One became pregnant after 10 cycles One showed erratic cycles

Each animal was exposed to a proven fertile male during day 9–14 of each cycle. The cycle was considered ovulatory if serum estrogen on day 8/9 was at least 200 pg/ml (range 200–960 pg/ml) and serum progesterone on day 18/19 was at least 2 ng/ml (range 2–9.6 ng/ml).

*Mean cycle length of 28 g Banjauri fed monkeys computed from 54 cycles was 29.2 \pm 4.4 days.

*Whereas groups IIA and IIB received treatment from days 1 to 14 of the first cycle, group IIC received treatment for days 1–10 of the first cycle.

50 g of Banjauri during post-partum period were not protected against pregnancy and all the animals became pregnant within 2 to 3 cycle exposures to proven fertile males.

To test the efficacy of Banjauri to block fertility of regularly cycling monkeys totally three different regimens were employed. Monkeys which were fed 1 g/day over 14 days ($n=4$) became pregnant within 3 exposures to males. However, monkeys which were fed 2 g/day for 14 days remained non-pregnant even after 22 (mean) exposures to proven fertile male monkeys (Table 5). The number of ovulatory cycle exposures varied from a minimum of 14 to a maximum of 25. Out of these cycles 65% were ovulatory as judged by serum estradiol levels on day 9/10 and progesterone levels on day 18 of cycle. In the group which received 5 g/day over a period of 10 days one became pregnant after six cycles, one conceived after 10 cycles while one exhibited very erratic cycles and as such breeding studies with this were discontinued. It was also noticed that the animals which were fed 2 g Banjauri/day did not cycle properly during later part of the study (i.e. 18 months after termination of feeding) as judged by serum steroid (E_2 and P_2) hormone levels. A comparison of the number of exposures needed for pregnancy in our colony revealed that while in the control monkeys an average of 2.2 cycle exposure is needed to become pregnant, in the monkeys fed Banjauri during the menstrual cycle the animals remained non-pregnant even after 20–22 cycle exposures.

Indian folklore has several claims of medicinal plants having contraceptive efficacy. The majority of the claims, however, have not been subjected to strict scientific verification. The present study reveals that the dried powder of *Vicoa indica* administered as suggested by the folklore, i.e. in post-partum monkeys is not effective in conferring protection against pregnancy during subsequent exposures to proven fertile males. The dose administered was based on that claimed to be effective in the human. However, the animals which were fed a total dose of 28 g during days 1–14 of menstrual cycle, remained non-pregnant even after 150 ovulatory cycle exposures. It should be emphasized that the colony data suggest that an average of 3 ovulatory cycle exposure is adequate for pregnancy establishment and the fertility index of the female colony is fairly high. Also of the total number of cycles exposed, only ovulatory cycle was considered and even this is 5 times more than the number required for the control and in spite of so many exposures the animals have remained non-pregnant. A similar effect was observed in animals fed Banjauri by intragastric deposition. In view of the fact that the administration of plant powder is effective only when fed during the menstrual cycle, it is possible that the

active principle in the plant induces some irreversible changes in the reproductive tract (fallopian tube or uterus) resulting in infertility. Although this is only a preliminary study providing evidence only for the efficacy of the plant and not identifying the active principle, we feel that this observation needs attention in view of the possible potential as safe oral contraceptive. It is pertinent to note here that in a recent clinical trial conducted with Banjauri on 362 lady volunteers who were given 50 g of powder in a single dose on 2nd day of abortion along with water and eleven black pepper seeds only 14 cases of drug failure were reported⁵. It is currently unclear if the active principle of this plant product is affecting implantation or early embryonic development in the fallopian tube itself. Three compounds (sesquiterpene lactones) designated as Vicolide A, Vicolide B and Vicolide C have been isolated and characterized from the plant *Vicoa indica*. Of these, vicolide B was found to have antifertility activity in Wistar rats when administered at a dose of 100 mg/kg body weight⁶ and this was ascribed to the compound having antiestrogenic activity⁷. The possibility of these compounds bringing about occlusion of the fallopian tube has also to be considered. In this connection, the ability of quincarine to bring about occlusion of the fallopian tube may be of significance⁷. Considering that this plant extract provides extended protection against contraception following ingestion for one cycle only, it is felt that further controlled studies with the crude plant extract and few of the vicolide derivatives in non-human as well as human primates are warranted.

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Plantlet regeneration from leaf explants of oil palm seedlings

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Plantlet development was achieved from leaf explants of 18-month-old *dura* and 6-month-old *tenera* oil palm seedlings. Callus induction was noticed after 100–120 days in *dura* and 150–180 days in *tenera* on culturing in half strength Murashige–Skoog basal medium supplemented with 25 mg l⁻¹ 2,4-dichlorophenoxyacetic acid. Seven per cent of the cultured explants in *dura* and 10% in *tenera* produced embryogenic calli. These were transferred to regeneration medium containing 1 mg l⁻¹ zeatin riboside. Plantlet development from calli was achieved through both somatic embryogenesis and organogenesis. Histological studies on developmental stages were also reported.

CLONAL propagation of oil palm has commercial importance¹⁻³. Being a recalcitrant tree crop, the success of micropropagation of oil palm is chiefly related with the choice of explants; tissues from juvenile trees are found to be more responsive⁴. The tender leaf tissues of oil palm seedlings offer good source of explants for reasons of easy availability and sampling. Micropropagation will enable large-scale production of *tenera** hybrids from the performance tested *Dura* × *Pisifera* crosses. Here we describe the various stages of plantlet regeneration by using tender leaf explants of 18-month-old *dura* and 6-month-old *tenera* seedlings.

The sprouts of *dura* and *tenera* were collected from the Central Plantation Crops Research Institute, Regional Centre, Palode and maintained as pot cultures at CPCRI, Kasaragod. The *dura* seedling sampled for the present study is from the cross between 3D × 266D and the *tenera* hybrid from 333D × 609P. The seedlings were sampled destructively by removing the outermost leaves and retaining a few interior leaves with the middle column and surface sterilized by alcohol flaming inside a laminar flow. Subsequently, the outer leaf whorls were removed and only the central portion with meristematic region was used for culturing. Leaf lamina and leaf base were cut into small pieces (0.5 to 1 cm). A total of 60 leaf explants of *dura* and 30 leaf explants of *tenera* were initially inoculated into callus induction medium.

The details of the culture media are given in Table 1. The cultures in the callus induction medium were first incubated in controlled conditions (temp. 27 ± 2°C; rela-

tive humidity 55–60%) in dark till callus induction was noticed. Subsequently, the cultures were maintained in illuminated conditions with 16 h photoperiod (2500 lux) for further differentiation. Subculturing was done at monthly intervals.

The specimens selected for histological studies included developmental stages of somatic embryos and meristemoids. These were fixed in Carnoy's B fixative (60% absolute alcohol; 30% chloroform; 10% acetic acid) for 24 h and were dehydrated in alcohol–butanol series before embedding in paraffin wax. Serial sections of 10 µm were taken using a microtome. After deparaffinization, they were stained with 0.1% toluidine blue (TB), periodic acid Schiff's reagent (PAS) and mercuric bromophenol blue (MBB) respectively for the detection of total nucleic acids, total insoluble polysaccharides and total proteins^{5,6}.

Nodular calli were produced from leaf veins after 100–120 days of inoculation in *dura* (Figure 1) (4/60 cultures), while it took 150–180 days in *tenera* (3/30 cultures). No direct embryogenesis was observed. The actively dividing cells in culture medium are dominated by callus-forming habits⁷ and so was the case with the leaf explants excised from the basal parts. On transfer to the somatic embryogenesis/organogenesis medium the primary calli regenerated into somatic embryos and fast growing calli (Figures 2 and 3). The frequency of various developmental stages is shown in Table 2. Compared to *dura*, the regeneration process was slow in *tenera*. In this case the supplementation of zeatin riboside to the medium was delayed for 50–60 days and might have slowed down the regeneration process. The quantity of calli and the regeneration capacity were found to reduce on increasing the interval between subculturing (45–50 days). Somatic embryos developed into complete plantlets in the regeneration medium (Figures 4 and 5) whereas, fast-growing calli directly gave rise to a large number of meristemoids (Figures 6 and 7). Shoot development from meristemoids took 90 to 100 days. It may be seen that a larger share of plantlets was derived through organogenesis than somatic embryogenesis. However, plantlet regeneration through somatic embryogenesis with a minimum callus phase was also reported in oil palm⁸. Shoots with 3–4 leaves and having a height of 10–12 cm were subsequently transferred to rhizogenesis medium. Sufficient roots were produced (Figure 8) within 3–4 weeks. In some cases, filter paper bridges were provided to prevent the complete immersion of shoot for a long time. Plantlets with balanced roots and shoot were obtained within 60–80 days of culturing in rhizogenesis medium (Figures 9 and 10) and were transferred to pots.

At the time of transferring to pots, the plantlets were treated with Bavistin (1%) and thereafter with IBA solution (1000 ppm) for an hour. The potting mixture

*Based on the fruit structure, oil palm is classified as *Dura* (thick shell; less mesocarp), *Pisifera* (shell less; embryos rarely formed) and the commercially cultivated *Tenera*, the D × P hybrid (thin shell; more mesocarp (60–95%), with high oil content).

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Table 1. Culture media for callus induction, somatic embryogenesis/organogenesis and rhizogenesis

	Callus induction	Somatic embryogenesis/organogenesis	Rhizogenesis
Macro nutrients	$\frac{1}{2}$ MS	$\frac{1}{2}$ MS	$\frac{1}{4}$ Y3
Micro nutrient	$\frac{1}{2}$ MS	$\frac{1}{2}$ MS	$\frac{1}{4}$ Y3
Vitamins	$\frac{1}{2}$ MS	$\frac{1}{2}$ MS	$\frac{1}{4}$ Y3
Hormones			
2,4-D	25 mg/l	0.1 mg/l	—
2-iP	3 mg/l	3 mg/l	—
Zeatin riboside	—	1 mg/l	—
IBA	—	—	5 mg/l
NAA	—	—	1 mg/l
Ads	40 mg/l	40 mg/l	—
Thiamine	2 mg/l	2 mg/l	—
Casein, enzymatic hydrolysate	500 mg/l	500 mg/l	—
Sucrose	3%	3%	2%
Phytigel	0.2%	0.2%	—
Charcoal	0.25%	0.15%	0.1%
pH	5.7	5.7	5.7

IBA: Indole-3-butyric acid; 2,4-D: 2,4-dichlorophenoxyacetic acid; NAA: α -naphthaleneacetic acid; MS basal medium¹⁴; 2-iP: 6- γ -dimethylallylamino purine; Ads: Adenine sulphate; Y3 medium¹⁵.

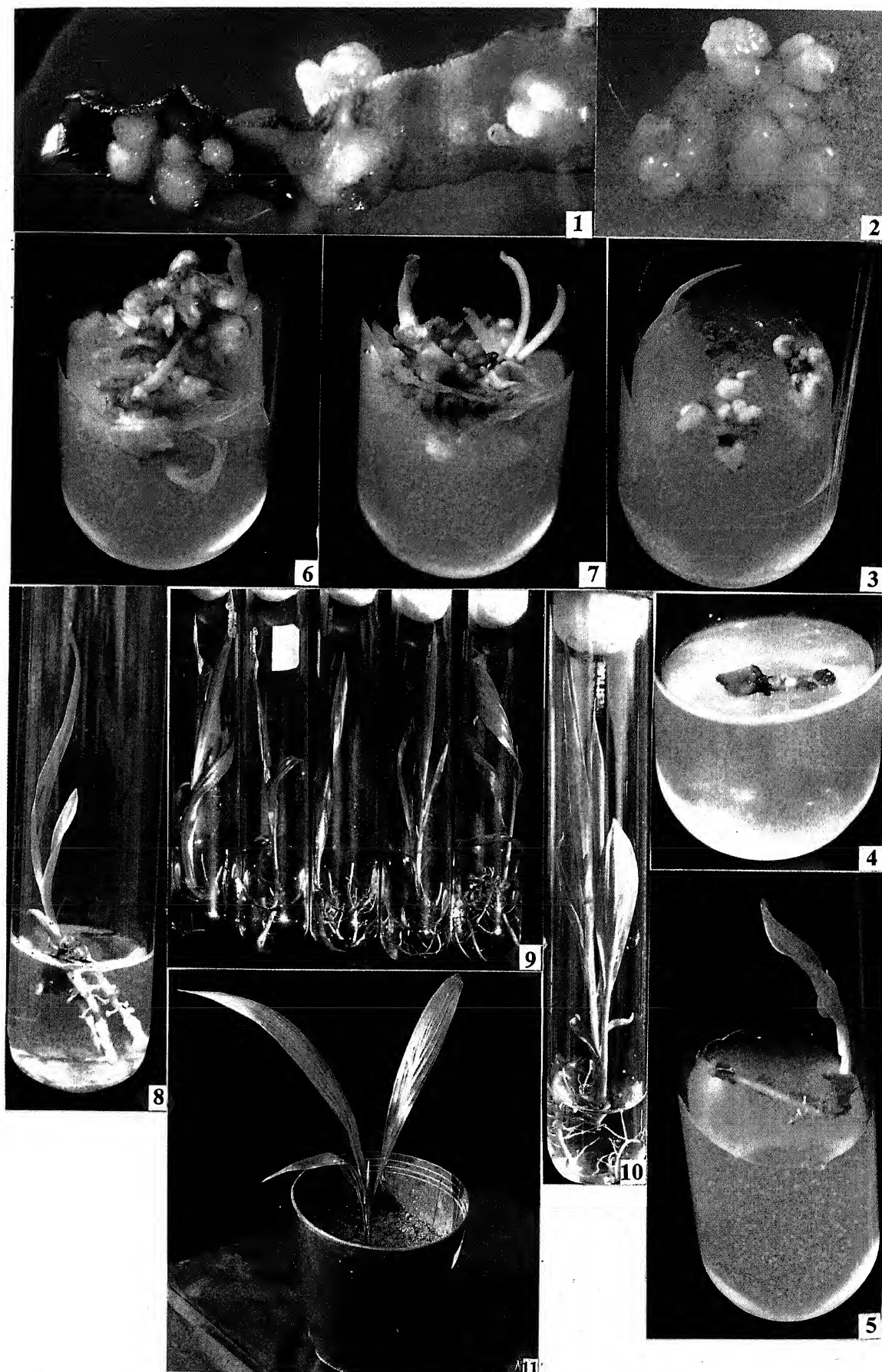
Table 2. Frequency of various plantlet regeneration stages at different periods of time (one out of 4 cultures in *dura* (D) and one out of 3 cultures in *tenera* (T)) in the regeneration medium

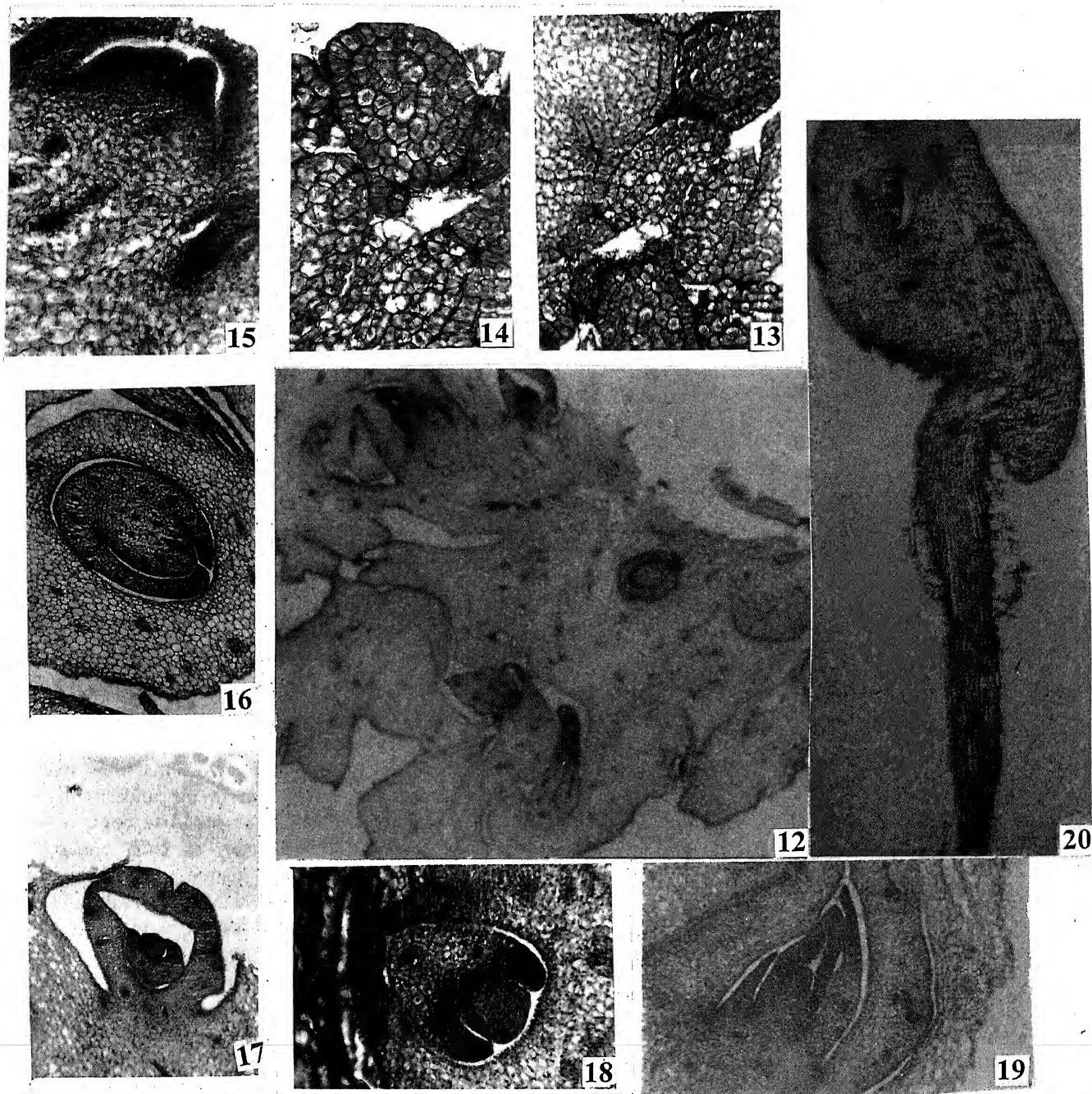
Days after initial inoculation	Somatic embryogenesis				Organogenesis				Total output of plantlets (cumulative no.)	
	No. of embryos formed		Total output of plantlets (cumulative no.)		Fast-growing calli subcultures		Shoots separated			
	D	T	D	T	D	T	D	T	D	T
200	14				25					
230	31		7		77					
250	8	3	7		178	25	175			
270	19	17	36	2	232	158	196	55		
300	88	30	42	15	344	243	416	162	98	40
400	192	46	121	24	754	280	792	324	560	74

used was sterilized with autoclave soil, sand and coir dust in equal portions. Initially, high humidity was provided to the plantlets by covering them with polythene bag; humidity was then gradually reduced by providing perforation to the bags and later by removing the bags during night. After 4 weeks, the bags were removed completely. The *ex vitro* establishment of plantlets was observed to be satisfactory (Figure 11). After 18 months from the start of the experiments, there were 175 plantlets established in pots. Comparable results were observed on repeating the experiments in three *dura* seedlings and two *tenera* seedlings.

Histological studies were done in *dura* palm cultures and the following observations were made. The primary calli were nodular and originated from the perivascular region of the vein, similar to the type obtained by

Schwendiman *et al.*⁹ in oil palm. These adventitious nodules could be easily separated from each other at the fragmentation lines (Figure 13). This physical isolation of calli from the explants is a prerequisite for somatic embryogenesis¹⁰. The fragmentation lines that demarcate the meristematic locus were also reported in the case of date palm¹¹ and oil palm¹². The nodular structures derived out of pale yellow coloured calli that tend to differentiate were found to be rich in nucleic acids (Figure 13). Cell differentiation was found to be very rapid in these fast-growing calli. In this stage, a combination of increased divisions and decreased differentiation confers a meristematic state on the cell nodules, which is an essential prerequisite for embryogenic competence of its cells¹³. It was observed in the present study that the origin of somatic embryos/mer-





Figures 12-20. Ontogeny of organogenesis/somatic embryogenesis from *dura* oil palm leaf explant. 12, Section of 5 mm sized fast-growing calli showing 5 shoot meristems (100 \times). 13, Section of embryogenic calli showing few embryos cut in oblique section (400 \times). 14, An enlarged globular embryo with actively dividing large sized, dense cytoplasmic cells with small haustorium (400 \times). 15-18, Serial sections of meristemoid and shoot development from fast-growing calli. 19, LS of shoot meristem with 4 leaf whorls (400 \times). 20, LS of germinated somatic embryo with shoot and root (PAS) (100 \times).

Figures 1-11. Somatic embryogenesis/organogenesis from *dura* (3D \times 266D) oil palm leaf explant. 1, Callus induction after 100-120 days in 25 mg/l 2,4-D, 3 mg/l 2-iP, and 40 mg/l adenine sulphate on half strength MS medium. 2, Friable calli separated from the leaf explant in regeneration medium with zeatin riboside 1 mg/l. 3, Formation of somatic embryos as well as fast growing calli in regeneration medium. 4, Germination of separated somatic embryo. 5, Somatic embryo developed into plantlet. 6 & 7, Fast-growing calli after 4 weeks in regeneration media gave large number of meristemoids and multiple shoots. 8, Formation of roots in rhizogenesis medium. 9 & 10, Plantlets with balanced roots and shoot are ready for transferring to pots. 11, Established plantlet in pot.

istemoids was from a group of cells (multicellular) (Figure 13). The proembryogenic nodules were found to be present towards the periphery of the calli, the rest of the cells in the calli probably acting as nurse tissue to the emerging embryoids. These observations are in confirmation with those made by Schwendiman *et al.*⁹.

Continued meristematic activity was observed in the calli which led to the formation of a central zone of the embryonic shoot apex (Figure 14). Subsequently, the apex became well defined as a rounded dome (Figures 15–17). After 6 weeks of culture in the regeneration medium, green-coloured structures were observed on the surface of the calli. All these structures were highly vascularized and they play the role normally attributed to the haustorium by taking up nutrients from the culture medium. Cross sections of these structures showed a large number (10–25) of meristemoids and shoot primordia (Figure 12). A representative series of developmental stages is shown in Figures 14–20.

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Differentiating conductive and resistive inhomogeneities: A new approach in groundwater exploration

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Lateral inhomogeneities, such as dykes or shear zones give rise to false low-resistivity layers in vertical electrical sounding curves which are likely to be misinterpreted as a water-bearing zone at depth. In groundwater exploration it is necessary that such anomalies be identified, i.e. a near-horizontal discontinuity, a conductive (probably water-bearing) lateral inhomogeneity or a resistive (e.g. a dyke) vertical feature. Lateral effects can be distinguished from those due to depth effects by offset soundings or crossed azimuth soundings, while conductive lateral inhomogeneities can be differentiated from resistive ones by a modified azimuthal sounding technique. Under favourable geoelectric conditions it may be possible to determine the direction and amount of dip of the vertical feature as well so that well sites could be located at a suitable distance in the down dip direction of the feature.

In the predominantly crystalline rock terrains of south India, groundwater occurs in two distinct zones, namely the near-surface weathered and decomposed rock material (regolith) and the joints and fractures that may extend to a few hundred metres depth in the underlying bed rock. Shear zones are also often found to contain substantial amounts of groundwater, as such zones are generally highly fractured and jointed, a condition conducive for storage and movement of groundwater. Over-exploitation of groundwater in several parts of southern India has resulted in the drying up of the weathered rock horizon, restricting the availability of groundwater to the fractured rock zones, which extend to relatively deeper levels. The success of a borewell under these conditions depends on the presence of such deep water-bearing zones, and therefore, it is essential that they be identified to successfully locate sites for constructing water wells.

Availability of groundwater in these fractures, joints, shear zones, etc. is facilitated by weathering of the parent rock along them, increasing their porosity, and hence, water holding and transmitting capacity. It must be emphasized here that below a certain depth the weathering of the parent rock is confined mainly to the joint planes of the fracture and shear zones, leaving the bulk of the rock body un-altered and relatively fresh. The weathering renders such zones electrically more conductive in comparison to the country rock, and hence, at first glance, it may appear to be an easy target for

exploration through geo-electric techniques. However, these weak zones in the country rock, often having narrow widths and with dips ranging from vertical to near-horizontal, are likely to be missed during investigations. But, in view of the importance of identifying them for a successful groundwater development programme, detailed investigations were carried out to develop a suitable exploration technique. From these studies, a field methodology has been evolved to identify vertical and near-vertical features, differentiate them as geo-electrically conductive or resistive and then approximately determine the direction and dip amount of these lateral inhomogeneities.

The vertical and near-vertical joint/fracture zones, narrow shear zones, dykes and veins can essentially be considered as lateral inhomogeneities in the country rock. Their presence in an area may be recognized by the resistivity anomalies they generate, provided that these inhomogeneities are sufficiently wide, and also that adequate resistivity contrast exists between them and the country rock. Resistivity profiling using alpha-, beta- and gamma Wenner arrays^{1,2} or vertical electrical soundings (VES) such as Barker's³ offset Wenner soundings and crossed azimuth soundings⁴ are helpful in identifying lateral inhomogeneities. However, these techniques are used only when one is specifically looking for the presence of lateral inhomogeneities and rarely

otherwise. In groundwater exploration using resistivity methods, the normal practice is to carry out a few vertical soundings in the area of interest and select the best out of them for constructing wells. The problem is therefore to identify the presence of vertical features, if and when present, from an analysis of these VES curves without resorting to the above additional techniques. It is known that a lateral inhomogeneity, be it resistive or conductive, gives rise to a false low-resistivity geo-electric layer in the VES curve, when one of the current electrodes is in contact with it^{4,5}. A low-resistivity layer in a VES curve may also be caused by a relatively more conductive lithological unit at depth. The problem is shown in Figure 1, wherein three instances of diverse geological conditions giving rise to essentially identical VES curves are shown. This underlines the need for a technique to differentiate between the causes of these anomalies.

The objective of identifying a lateral inhomogeneity is achieved by the so-called offset sounding method described by Ballukraya *et al.*⁴, wherein a series of closely spaced soundings are carried out along a common electrode spread direction. Schlumberger electrode array has been found to be preferable for this purpose as it minimizes errors in the measured values that may be introduced by the shifting of potential electrodes for each and every measurement, as with Wenner electrode

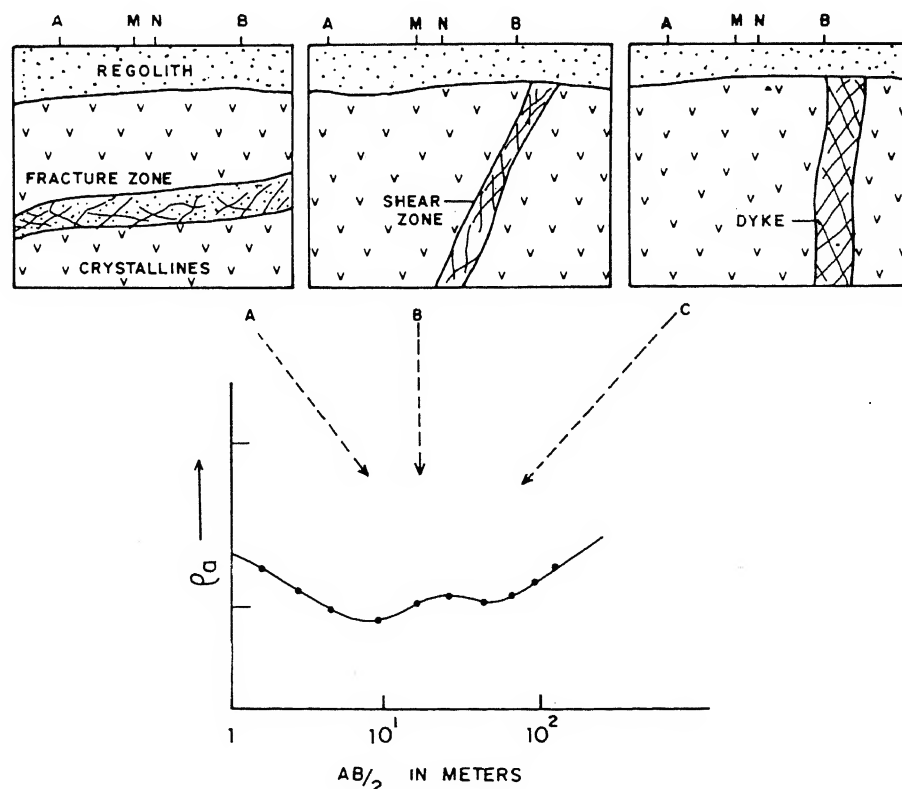


Figure 1. Shape of the VES curves that may be obtained over three different geological sections.

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configuration. The presence of a lateral inhomogeneity will be generally reflected in the form of a false low-resistivity layer in the ascending part of the VES curve. Once such an anomaly is noticed, two or more soundings are carried out, close to each other, along the same electrode spread azimuth at distances of about 10 to 15 m from each other. If the low-resistivity layer noticed in the VES curve is caused by a horizontal discontinuity (Figure 1a) at some depth, the current electrode separation (AB) at which the curve starts its

downward trend will essentially be the same in two or more of the offset VES curves. On the other hand, if it is due to a lateral inhomogeneity (Figure 1b and c), the electrode separations at which the low-resistivity layer begins will be offset by the respective distances between the sounding stations. The AB separation at which the low-resistivity layer occurs in these VES curves will thus clearly indicate its cause (lateral or horizontal) and its location (distance or depth).

Figure 2 shows a set of four VES curves obtained

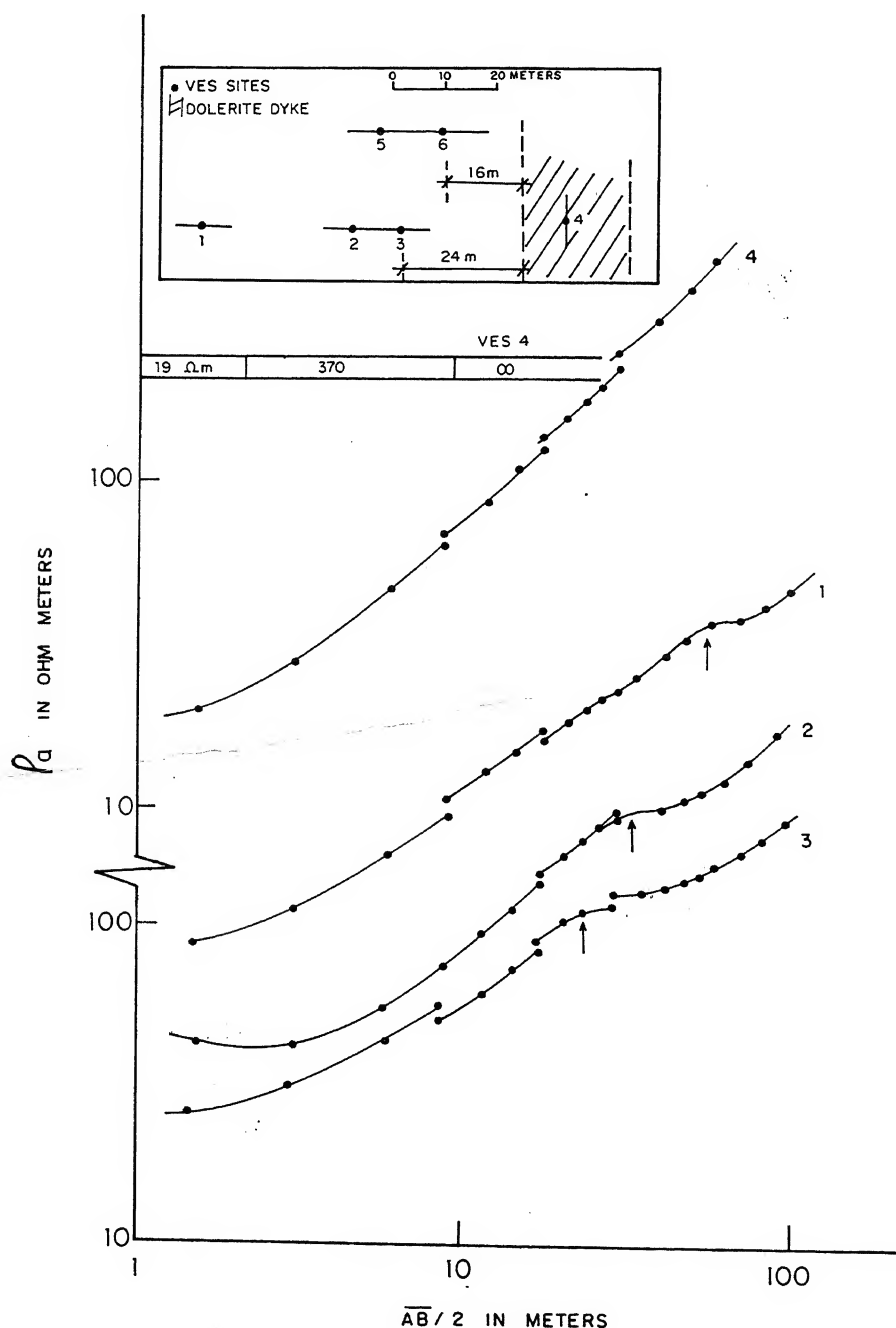


Figure 2. Effect of a resistive lateral inhomogeneity on VES curves.

near a dolerite dyke in Veerapuram village, Tamil Nadu ($12^{\circ}44'30''\text{N}$, $80^{\circ}0'05''\text{E}$). This 20 m wide dyke is found exposed for over one km length. Three soundings are to the west of the dyke, with electrode spread direction normal to the strike of the dyke. As seen from the VES curves, a low-resistivity layer is generated whenever one of the current electrodes crosses the dyke. The $AB/2$ separation at which the anomaly begins is equal to the distance between the VES site and the dyke-country rock contact. As observed from the figure, the low-resistivity layers begin at $AB/2 = 63, 33$ and 24 m respectively in VES curves - 1, 2 and 3, thus offset by

the inter-VES station distances (9 and 30 m). This clearly illustrates the effect of a resistive lateral inhomogeneity on the shape of VES curves and the generation of a low-resistivity layer therein.

Once the presence of a lateral inhomogeneity is thus established, its strike direction can be found out by carrying out two or more sets of offset soundings along azimuths parallel to that of the first set, and joining the inferred contact zones (inset, Figure 3).

A lateral inhomogeneity thus identified can be any one of the features such as joint/fracture zone, dyke, vein, fault or shear zone. In geo-electric terms, it can

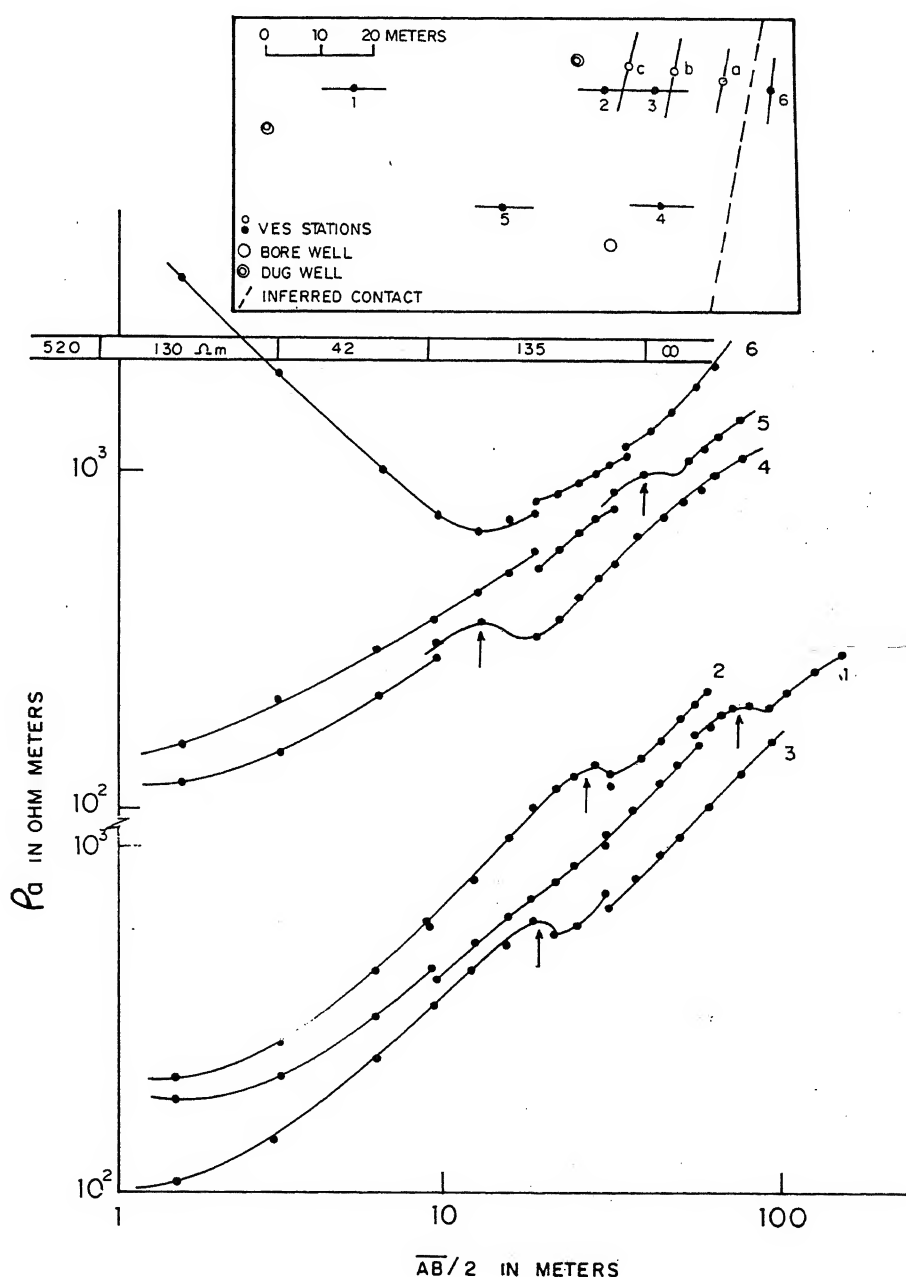


Figure 3. VES curves from Athur study area with an inferred conductive lateral inhomogeneity.

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be a resistive or a conductive layer in relation to the host rock. Both give rise to a false low-resistivity layer and differentiating the two in the field, on the basis of geoelectric parameters alone is generally difficult if not impossible. In groundwater exploration, however, it is essential that we establish the true nature of the inhomogeneity. For, a conductive zone may be water-bearing, hence the target of exploration, while a resistive body could be a massive dyke, to be avoided while locating well sites. The problem is solved by carrying out another set of soundings, but this time over the inferred inhomogeneity and with the electrode array parallel to the strike direction of the inhomogeneity. These VES curves, obtained at stations located directly over the inhomogeneity, are likely to indicate the nature of the body, by the presence or absence of geo-electric layers corresponding to relatively thick, decomposed or weathered rock zones. A resistive body (say, a massive dyke) will normally have a very thin weathered rock layer, whereas a conductive body (say, a water-bearing fracture or shear

zone) will have a comparatively thicker weathered rock layer at the top. Thus, VES curve-4 in Figure 2 shows a three-layer section with very thin weathered rock layer as expected over a dolerite dyke. VES curve-6 in Figure 3 on the other hand, shows a five-layer section, with substantially thick overburden as well as weathered and partly weathered rock horizons, indicating that underlying it is a comparatively conductive zone. This is helpful in differentiating a resistive from conductive lateral inhomogeneity.

It is possible that the lateral inhomogeneity is inclined in which case the direction and amount of the dip have to be estimated to locate the well site at a suitable distance away from the surface trace of the inhomogeneity. The ground distance to a favourable location in the down dip direction will depend on the dynamic water levels prevailing in the area—deeper the levels, larger the distance to the prospective well site for a given dip.

An empirical method of estimating the dip of the

Table 1. Interpreted results of VES curves from Athur area

VES no.	Layer resistivity (Ωm)					Layer thickness (m)			
	ρ_1	ρ_2	ρ_3	ρ_4	ρ_5	h_1	h_2	h_3	h_4
1	170	1020	Very high			2.1	15.8		
2	200	1800	Very high			2.1	9.5		
3*	100	3500	?			1.8	?		
4*	105	420	2500 (?)			1.9	6.3	?	
5*	190	570	3870	?		1.5	7.2	?	
6	520	130	42	135	High	0.9	2.1	5.6	32.8

*Values approximate/interpretation incomplete.

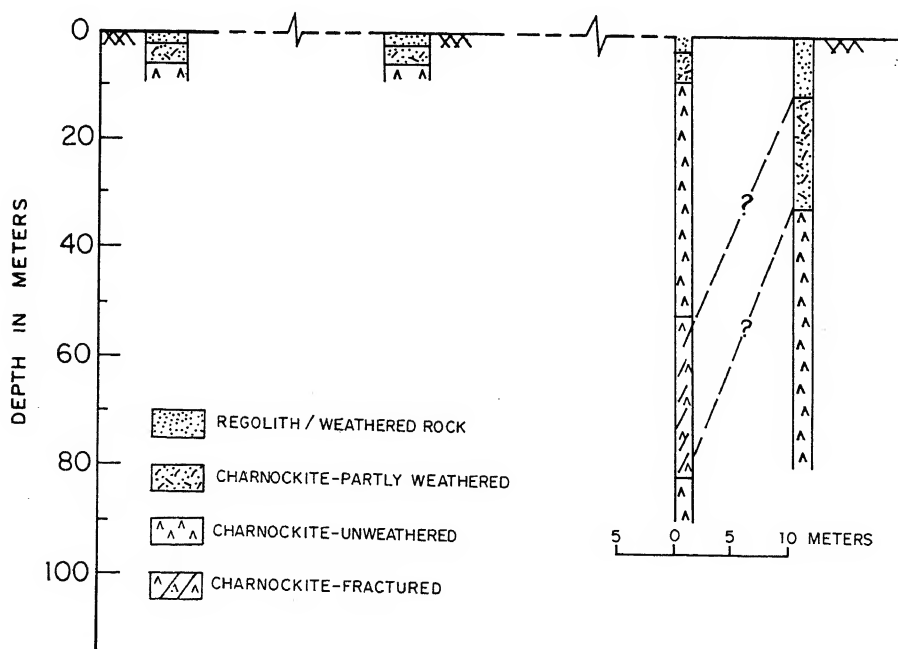


Figure 4. Subsurface geology of the Athur study area showing steeply dipping fracture/shear zone.

vertical feature is by conducting a series of soundings, in a direction normal to the strike of the inhomogeneity and with the electrode array parallel to the strike direction (Figure 3 a,b,c). A conductive zone dipping in the direction of these soundings is likely to give rise to a low-resistivity anomaly in the VES curves, and from the depths at which they are indicated in two or more VES curves, the approximate amount of dip can be estimated. Though dipping bodies can be recognized by theoretical analyses of resistivity data⁶, the methods are not unambiguous nor can they be easily adopted in field. Therefore, the empirical method suggested above is far more advantageous.

The proposed technique was tested in the field near Athur town (11°34'40"N, 78°35'14"E) in Tamil Nadu. The area is underlain by charnockite rock with a thin regolith and weathered rock zone near the surface. Several vertical electrical soundings were carried out in a grid pattern using Schlumberger electrode array, in a small plot of land for the purpose of locating a site for the construction of an agricultural borewell. Six of the VES curves obtained from this are shown in Figure 3, along with their relative locations in the inset. The presence of a lateral inhomogeneity is indicated by the false low-resistivity layers in the offset VES curves 1, 2 and 3 and confirmed in VES curves 4 and 5 from a second profile. From the position of the anomalous low-resistivity geo-electric layers in each of the VES curves, the contact of the inhomogeneity was fixed at distances of 75, 27 and 18 m respectively to the east of stations 1, 2 and 3 along the first profile and 12 m from station 4 along the second profile. The approximate strike of the inhomogeneity was established by connecting the contacts along the two profiles (inset of Figure 3). In the next step, VES-6 was carried out over the inferred inhomogeneity with the electrode spread direction parallel to the strike of the anomalous body. The results of the analysis of these six VES curves, using Auxiliary Point Charts⁷ and refined by computer simulation are given in Table 1. The quantitative interpretation is partial and approximate in the case of VES curves influenced by the effects of the lateral inhomogeneity. A marked difference is observed in the geoelectric sections indicated by curves 1 to 5 and that of curve 6. While a thick overburden ($h_1 + h_2 + h_3 = 8.6$ m) and a comparatively thick partly weathered rock layer ($h_4 = 32$ m; $\rho_4 = 135 \Omega\text{m}$) is indicated from VES-6, the depths to bed rock layer at stations 1 to 5 are much smaller, less than 2 m on an average. This sharp contrast indicates the existence of a conductive lateral inhomogeneity in the area of VES-6 which could be interpreted as a water-bearing shear/fracture zone.

The sub-surface lithology as observed from the dug well sections in the area (Figure 4) confirms the geological interpretation of geoelectric data in respect of

VES stations 1 to 5. Two borewells were drilled, one at station-6 and the second at a distance of 12 m, from station-6, towards station-3. The location of the second well was on a trial basis. The lithological sections of the two borewells are shown in Figure 4 and the drilling of the second well provided useful information.

Both borewells yielded substantial quantities of groundwater, 95 lpm (litres per minute) at VES-6 and 140 lpm from the second well. Of greater interest, however, is the lithological section thrown up by the two borewells. At VES-6 a thick overburden (10 m) is present underlain by a 24 m thick partly weathered rock zone, followed by the bed rock. In the second well, fresh bed rock was encountered at a relatively shallow depth of 9 m. In addition, a fractured rock horizon extending from 49 m to 78 m depth was also encountered in this second borewell. Much of the groundwater in case of the first well is from the weathered rock zone between 25 and 34 m, while in the second well it is mainly from the fractured rock zone, extending between 49 and 78 m depth. It has been reported that in the summer months, following the commissioning of the wells, the discharge from the first borewell reduces sharply while that from the second well sustains to a large extent. The drill cuttings obtained from the fractured rock zone in the second borewell showed distinctive polished surfaces as well as microscopic fractures, indicating that it may be a shear zone, steeply dipping from the area of VES-6 towards VES-3. The interpretation of the geoelectric data as to the presence of a conductive lateral inhomogeneity has been thus proved correct. The higher yield and its sustained nature, in the second borewell are due to the shear zone being at a deeper level, and thus remaining fully saturated even in summer compared to the near-surface aquifer zone in borewell-1. This also underscores the need for determining the dip of the lateral inhomogeneity to locate the well site at an optimum distance from the surface contact.

The methodology suggested in this paper for identifying water-bearing zones in areas underlain by crystalline rock formations is a simple, field-friendly yet effective technique, which can be applied in groundwater investigations quite successfully.

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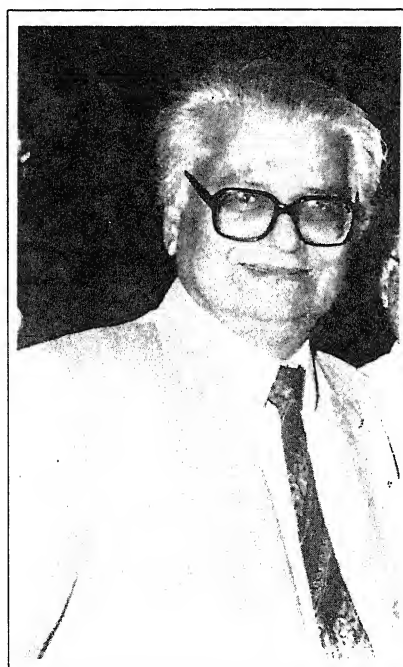
An obituary

B. K. Bachhawat, one of India's leading biologists, passed away peacefully on 23 September 1996. He was immersed in scientific activity till the very end.

Bimal Bachhawat was born on 26 August 1925 at Calcutta in a large family living modestly and consisting of five brothers and three sisters. All his brothers rose to high academic and social positions in the country. His tenacity and determination for education can be gauged from the fact that he used to swim across the river Hooglee to go to school. With a Master's degree in applied chemistry from Calcutta and inspired by B. C. Guha's work in biochemistry, Bachhawat obtained his Ph D degree in 1953 from the University of Illinois. Subsequently he worked at the school of Biological Chemistry, University of Michigan, Ann Arbor, where he discovered HMGCoA lyase with M. J. Coon—a fundamental contribution to the understanding of the formation of ketone bodies in mammals.

In 1957 he joined the Christian Medical College, Vellore where he established an internationally recognized school in the area of neurochemistry and glycobiology. Bachhawat began his studies on complex and difficult problems such as glycolipids, glycosaminoglycans and glycoproteins *vis-à-vis* neural development and neurological disorder. His efforts fructified in a short duration of few years only when he and his colleagues showed that the glycolipid storage disease metachromatic leucodystrophy is caused due to the absence of the enzyme arylsulphatase A. Until then the molecular basis for about 50 glycolipid storage disease had defied understanding. Soon this pathbreaking study set the pace for the elucidation of the enzymatic defects in other glycolipid storage diseases such as Gaucher's disease and Tay-Sachs disease, which led not only to the development of their prenatal diagnosis, but also to strategies for treating such genetic afflictions. His group also elucidated the mechanism for the biosynthesis and the degradation of cerebroside-3-sulphate, the lipid stored to abnormally high levels in the metachromatic leucodystrophy patients. Subsequently, his discovery of CMP-*N*-acetyl-

neuraminic acid was crucial to defining the turnover of *N*-acetylneuraminic acid at cell surfaces. His demonstration of glycosaminoglycans in neuronal development was also far ahead of its time just as the elucidation of the role of glycolipids as biological receptors was. He also pioneered sugar-bearing liposomes as a molecular trojan horse for the site-specific delivery of drugs and enzymes to the diseased organ only. He dedicated the



last decade of his life to the development of liposomal formulations for treating systemic fungal infections which have already benefited several human beings in the country. His research was respected widely—he was one of the most cited biologists of the country.

He was the recipient of numerous awards and honours which included Shanti Swarup Bhatnagar award (1962), Golden Jubilee Medal of IISc (1976), Birla Smarak Kosh (1986), FICCI award (1982), to name a few. He was honoured with the Padma Bhushan in 1990. He was elected to all the scientific academies in India and several of them conferred many honours and medals on him over

the years. He was the first Indian to be elected to the Presidentship of the Federation of Asian and Oceanian Biochemists (1983–85) and had the rare distinction of leading the Society of Biological Chemists twice as its President (1970–72 and 1990–94). During the latter period of his presidentship, he was responsible for successfully organizing the International Union of Biochemistry and Molecular Biology. He had also organized at least ten international conferences in India for the younger scientists to have the opportunity to interact with acclaimed authorities of their field. He was also deeply involved with ethical and socio-economical issues of human genome studies and was busy organizing an international symposium to be held in New Delhi in February 1997.

Bachhawat was a great builder of Institutions as is apparent from the setting up of the neurochemistry laboratory in 1957 at the Christian Medical College Hospital, Vellore—the first of its kind in the world until recently and the Department of Biochemistry at the University of Delhi. He was responsible for making the Indian Institute of Chemical Biology, Calcutta a leading centre in the area of contemporary biology in the country. He nurtured a large number of institutions in India and was untiring in his efforts to support younger scientists. He was an inspiring researcher with a clear mind and an elephantine memory. He was fond of daily discussions with his colleagues on latest developments in science. He was always compassionate and composed even during adversity. He would enthuse his colleagues beyond their expectations even when a string of experimental failures were narrated to him and for which almost always he would apportion blame to himself for his inability to anticipate the same.

He helped, advised and encouraged younger scientists throughout the country, influenced and hastened the advent of modern biological disciplines in the country. He had a very informal and unorthodox style of administration and anyone could meet him at anytime and often obtain instant help or solutions to their

problems. He could become a youngster in the midst of young people and enjoy their jokes with boisterous laughter. He took a great delight in inviting and feeding his students and friends.

A large number of his students have earned academic distinctions both within India and outside. Bachhawat excelled in bringing people from different disciplines together to facilitate generation of novel ideas. As the Chairman of the Technical Advisory Board (Biological Sciences) of the Council of Scientific and Industrial Research, for example, he organized a large number of brain-storming sessions, on topics such as on fats and oils, membrane biology, cell surface, drug delivery, protein engineering and molecular immunology. This, in turn, led to the establishment of the molecular immunology forum which meets once a year and which has given a great impetus to the research in this area in the country during recent years. His imprint on the agencies which foster the growth of biological sciences in India will be felt for a long time.

Bachhawat was fond of Louis Pasteur's 'in the field of experimentation chance

favours only the prepared mind' and the words of Frederick Gowland Hopkins 'the biochemist should remember that his data gain their full significance only when he can relate them with the activities of the organism as a whole. He should be bold in his experiments, but cautious in his claims. His may not be the last word in the description of life, but without his help the last word will never be said.'

It is indeed difficult to describe completely an outstanding humanist like him but his warmth, affectionate and adoring nature which knew no boundaries will be missed in abundant measure by his colleagues and students for a long time to come.

Bachhawat is survived by his wife Kamala, daughters Kalpana and Kiran and son Anand.

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BOOK REVIEWS

Advancements in Iron Nutrition Research. A. Hemantranjan, ed. Scientific Publishers, 5A, New Pali Road, P.O. Box 91, Jodhpur. ISBN: 81-723385-5. 1996. Price: Rs 650. 303 pp.

Agronomists and crop physiologists in India have focussed their attention since long on research in plant nutrition especially, on studies relating to mineral deficiencies in crop plants. These studies were mostly directed to the analyses of symptoms of the deficiencies.

However, little effort was made to investigate the physiological/biochemical basis of the deficiencies of macro- or micronutrients. As a result, in spite of many institutions in India working on mineral nutritions in crop plants in the past, there has been no cumulative effect on our understanding of the biochemical processes involved in mineral deficiencies in plants.

Of all the micronutrients, iron plays a vital role in plant growth and development. Fe-deficiency causes chlorosis, and this deficiency is uniquely related to the mobilization and uptake of iron by plants rather than its availability in the soil. The book *Advancements in Iron Nutrition Research* is primarily aimed at focussing this aspect of iron nutrition in plants. The editor has collected 12 review articles which mostly address soil-plant iron deficiency and control, plant root and soil contacts for iron mobilization, phytosiderophore production and siderophore-mediated iron uptake. Besides these, there are short reviews on the role of iron in symbiotic nitrogen fixation and also mycorrhizae-based iron uptake. The modes of iron-mobilization in 'graminaceous' and 'non-graminaceous' plants have been adequately emphasized. While the book contains some useful information on Fe-chlorosis, and on methods for amelioration of iron deficiency in legumes and other plants, it is neither comprehensive nor authoritative. The editor seems to have put little effort in having all the reviews in uniform style and format in presentation, avoidance of unnecessary repetitions and critical treatment of contents. While there are three articles on phytosiderophores (even though one relates to N_2 -fixing cyanobacteria), there is no in-depth discussion on the genetic and molecular-biological aspects of iron

transport across the membranes; regulations of haeme-proteins and iron-sulphur centres and flavodoxins and ferredoxin. Collecting a number of articles even though they are contributed by the leaders in the field, does not produce a good reference or a good textbook. The book *Advancements in Iron Nutrition Research* suffers from this inadequacy. The topic selected by the editor is an important one; it is quite topical. We need books that deal with plant nutritions for courses in soil biology, crop physiology, plant biology and biotechnology. Although this book contains some valuable information on iron nutrition relating to its mobilization and uptake by plants, it does not meet the needs of students and teachers or the requirements of researchers interested in basic and applied aspects of plant nutrition. The Scientific Publishers, Jodhpur, have been publishing a series of books on plant physiology and related topics. While the readers of plant sciences welcome such publications, it is quite disheartening to see poor quality of illustrations, inadequate indexing, besides printing errors.

In spite of these shortcomings, the book is a timely publication. As stated in the beginning, despite having had a lead in studying mineral deficiency symptoms in plants in India, we have lagged behind in focussing our attention to biochemical and genetic basis of the developments of iron deficiency-symptoms. We need to know the signal transduction mechanisms by which iron controls transcriptional and translational processes; iron-regulation of electron transfer and energy transduction processes. It is hoped that the readers of this book would get sufficient hint that use of cyanobacterial systems offers promises for making advances in these directions.

In short, the book therefore serves an useful purpose but it is rather costly. One hopes to see a copy of this book in college and university libraries.

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The Archaean and Proterozoic Terrains of Southern India within East Gondwana – Textbook and Field Guide. M. Santosh and M. Yoshida, eds. Gondwana Research Group Memoir No. 3, Field Science Publishers, Osaka, Japan. 1996. pages xii + 403.

Gondwana Research Group (GRG) was founded recently by Masaru Yoshida of Osaka University, Japan with active participation by M. Santosh of the Centre for Earth Science Studies, Thiruvananthapuram. The GRG has already published two memoirs (Memoir 1, *Granulites of India, Sri Lanka and Antarctica*, and Memoir 2, *India as a Fragment of East Gondwana*) which have made the geological community, particularly the granulite specialists, to sit up and take notice. The Memoir 3 of the series includes a textbook (Part I, 325 pp) and field guide (Part II, 78 pp) and is a contribution to the International Geological Correlation Programme's (IGCP, UNESCO, Paris) Project No. 368: Proterozoic Events in East Gondwana.

The textbook is organized into sixteen chapters, each chapter presenting a review of some aspects of the main theme. The volume commences appropriately with Mohana Kumar's historical account of the fascinating concept of Gondwanaland from Ptolemy to Yoshida. The linkage of southern India with the Gondwanic neighbours of Sri Lanka and Antarctica (Yoshida and Santosh) and with Madagascar (Windley and Razakamanana) is the next main topic. A brief summary of the deep continental crust of India is given by Mahadevan. Jayananda and Peucat have compiled the isotopic dates of southern India. Remote sensing studies of the Proterozoic, crustal scale shear zones in southern India have been outlined by Chetty. An overview of alkaline magmatism of southern India is provided by Rajesh and Santosh. Genesis of gold, gemstone, graphite and molybdenite in Kerala is discussed with modern laboratory data by Rajesh-Chandran.

A spate of metamorphic studies follows: Karnataka-Tamil Nadu transition zone (Janardhan and Anto), Dharmapuri area (Rameshwara Rao and Narayana), Nilgiri hills (Srikantappa), Madurai Block (Anand Mohan), Trivandrum Block (Chacko, Lamb and Farquar; Santosh;

Satish Kumar and Santosh). The volume concludes with an unrelated paper on evolution of Central Indian craton by Divakara Rao and co-workers.

The field guide describes ten excursion stops chosen on the basis of easy accessibility and availability of good amount of data. Sketch maps with up-to-date descriptions of state-of-the-art laboratory information enhance the value of the field guide, which is perhaps among the best produced in recent years.

The papers are heavily weighted in favour of thermobarometry including recent studies on fluid inclusions and stable isotopes. Geochronology is also a favourite topic. While the enormous output of laboratory data will be useful for interpreting geological evolution of the region, a broad regional map showing tectonic blocks with a brief write up would have provided a good backdrop for discussion. An instructive abstract with each paper will surely have helped a rapid reader, especially in the absence of a well-organized summary. The concluding remarks or summaries given in some papers are so sketchy as to serve the author's only purpose of somehow completing his piece. A large number of papers from Kerala, particularly from Santosh's group may add to the local flavour, but may affect the reach, unless it is more broad based. When we integrate all laboratory data, it may still be necessary to verify them through field relations for which one needs more field work, which does not sadly form a significant part of new research projects.

It is to the credit of the editors and authors that the drawings are neatly and attractively done. Generally, the photographs which substitute for textual description, are not reproduced well enough. Spelling and syntax mistakes could have been reduced by more rigorous proof reading. The reference list is up-to-date, exhaustive and useful to an avid researcher. Addresses of the authors if given in the paper itself would have been very helpful. On the whole it is a

valuable compilation, useful for professionals, teachers and students.

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Annual Review of Physiology 1996.
Joseph F. Hoffman, ed. Annual Reviews Inc., 4139 El Camino Way, P.O. Box 10139, Palo Alto, California, 04303-0139, USA. Vol. 58. Price: USA \$ 54, Elsewhere \$ 59. 815 pp.

The Annual Reviews have always been heavy reading. Each volume every year has contained several chapters; each chapter dealing with one particular branch of physiology – respiration, blood, digestion, nervous system, etc. The pattern of presentation is that each chapter is under the charge of a section editor. The section editor then decides on the particular small topic that seems to be of immense current importance in that particular branch of physiology. The current volume concentrates on molecular phenomena that affect the particular branch of physiology that is being reviewed. Thus for example, when the nervous system is being reviewed, in point of fact, the study of one specific ionic channel in the nerve cell membrane is reviewed. The whole volume is devoted to such specific molecular phenomena that are important to the various branches of physiology. Though this is an index of the importance of molecular biology in modern biological thinking, it does tend to give a very lopsided view of the subject concerned.

Given this limitation, one must view this Journal (*Annual Review of Physiology*), as meant for senior researchers, who are already working intensively in the field. Such people will find the annual reviews very useful and the reviews written by very erudite thinkers in the field. This particular issue will be of immense

value to those researchers who concentrate on molecular phenomena. Others will find the volume heavy reading and difficult to digest.

One aspect of the Annual Reviews requires special mention. The first chapter is always written by a senior researcher, who writes an informal biographical chapter, on the particular field that has been his absorbing interest throughout his life. The first chapter should be read by all youngsters (graduate or undergraduate), who intend to devote their lives to academic research. They will find themselves being introduced to the excitement and fascination of science. In this volume, the first chapter is written by H. E. Huxley. This is the man who introduced the concept of the 'sliding filament' in skeletal muscle contraction. He introduces the reader to his desire to study nuclear physics, explains why he became disenchanted with nuclear physics (the use of nuclear bomb in the Second World War), his introduction as a physicist to the study of X-ray diffraction and how he came to use this to define the molecular structure of the contractile elements of skeletal muscle. It exemplifies how a strict physicist can unravel biological secrets and positively direct the thinking of biologists. As far as this volume of the *Annual Review of Physiology* is concerned, this particular chapter by H. E. Huxley focusses the reader's mind onto the need for understanding many biological phenomena at the molecular level.

By and large, this journal contains reference material, useful to the senior researcher. It also contains one chapter that will inspire the youngster. This journal should be stocked by every library that deals with biology. Individuals should only buy those volumes that are of particular interest to them.

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GOVERNMENT OF INDIA

DEPARTMENT OF SCIENCE & TECHNOLOGY

Programme on Nonlinear Phenomena, Complex Matter and Biologically Inspired Physics

The importance of nonlinear science, soft condensed matter (such as colloids, powders, polymers and membranes) and biologically inspired physics has emerged very clearly in recent years. In view of this and given the large mutual overlaps of these disciplines as well as their interdisciplinary nature, the DST has considered it fit to constitute a separate Programme Advisory Committee (PAC) specifically to foster research in these areas. Suitable project proposals are invited in the above general fields.

Research proposals should pay particular attention to the development and application of ideas and techniques that cut across disciplines and/or seek to elucidate and provide insight into the general paradigms of complexity and nonlinearity.

Proposals that come under this general purview include:

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Physics of soft condensed matter. Membranes (biological and model), Langmuir monolayers and Langmuir-Blodgett films, micelles, vesicles, lamellar structures, microemulsions, emulsions, foams, and other structures in surfactant solutions: liquid crystals, both thermotropic and lyotropic, polymer solutions, gels, and melts: colloidal suspensions, including ferrofluids, electro-rheological fluids, etc.

Biologically inspired physics. Models in evolutionary biology and population genetics, evolution of complexity, neural networks, optimization, protein folding, motor proteins and molecular ratchets, etc.

Qualified researchers interested in submitting project proposals to be considered for funding may write to the following address for further details:

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PAC on Nonlinear, Complex and Biological Systems
SERC Division
Department of Science & Technology
Technology Bhavan
New Mehrauli Road
New Delhi 110 016

CURRENT SCIENCE

A fortnightly journal of research
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INDO-AUSTRALIAN SCIENCE & TECHNOLOGY COLLABORATION PROGRAMME

Department of Science & Technology (DST), Government of India invites applications from scientists for financial support under the Indo-Australian Science & Technology Research Programme for exploratory visit of up to 14 days with an objective to develop joint research projects in the areas of Medical Science and Human Nutrition; Food Technology; Energy (including Renewable Energy); Environmental Management and other theme areas which might meet national interest.

2. While considering proposals, particular importance will be given to the scientific gains for India & Australia and where these could be applied to industry. Preference will be given to the proposal which has the consent of both Indian and Australian partners and is likely to result in a joint research project for a period of 2-3 years.

3. Financial support will be limited to return international airfare travel from DST. The Australian Department of Industry, Science & Tourism (DIST) would provide A\$ 150/- per day towards accomodation, boarding and out of pocket expenses to the visiting Indian scientists under this programme. Funding for equipment, bench fees, institutional overheads, etc., will not be provided under this programme. Application forms along with guidelines for such collaboration may be obtained from the following address:

Shri S. K. Varshney, Senior Scientific Officer, International Division,
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E-mail: apk@alpha.nic.in. or apk@udst.sirnetd.ernet.in.

4. Requests for applications may be made latest by **15 February 1997** alongwith a *self-addressed unstamped envelope*. The visit proposal completed in all respects should be submitted latest by **28 February 1997**.

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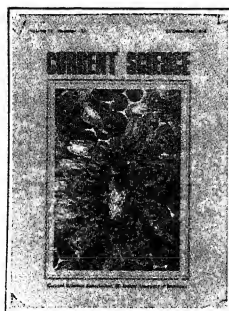
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COVER. The queen and her 'court' – a honey bee queen surrounded by a retinue of worker bees who lick and feed her and while doing so receive and redistribute pheromones produced by her (Photo by Kenneth Lorenzen, University of California, Davis). What makes the queen special appears to be a keto group in the major component of her pheromone blend. See page 975.

Indexed in CURRENT CONTENTS/GEODATABASE/CHEMICAL ABSTRACTS

In this issue

Chemistry, caste and honeybees

Does chemistry determine behaviour? Undoubtedly so, but rarely do chemists and behavioural biologists find a common meeting ground. In a speculative paper in this issue, Gadagkar (page 975) asks a provocative question, 'What is the essence of royalty' that determines the queen in a honey bee colony? The answer appears to be provided by a recent paper by Plettner *et al.* (*Science*, 1996, 271, 1851-1853), which examined the mandibular gland secretions of queen bees and workers. Interestingly, the secretions which contain hydroxylated, unsaturated fatty acids differed only in the position of the carbon atom that is hydroxylated. The differences arise because of a 'caste-specific bifurcated pheromone biosynthetic pathway' for the production of these compounds. The major pheromone produced by queen bees is 9-keto-(E)2-decenoic acid (9-ODA), a compound with a name far more formidable than its chemical structure. This substance differs from the major component of the worker pheromones, which lack the carbonyl group at position 9; tempting Gadagkar to state emphatically that the 'essence of royalty is just one keto group'.

Caste-specific biochemistry in social insects like honey bees provides a chemical rationalization of the differentiation between the queen and workers in a colony. The remarkable phenomenon of colonies that contain 'thousands of sterile workers, a few hundred drones and a single fertile female, the queen' has fascinated sociobiologists for a long time. Several questions remain to be addressed,

the most obvious being - who came first, the queen or the worker? In considering this problem Gadagkar returns to the reactions of pheromone biosynthesis and argues that pathways in workers appear to be more 'ancestral' while that in queens which result in 9-ODA appear to be more 'derived'. His thesis is based on the argument that the production of the keto acid (9-ODA) is 'energetically unwise', thereby suggesting a more important motivation than mere production of energy by oxidative metabolism. There are many fascinating issues to be considered, not the least of which is the evolution of sociality. Queen bees can indeed 'be thought of as an invention of sociality'.

Gadagkar uses this essay on the essence of royalty to emphasize that the failure to make distinctions 'between proximate physiological explanations and ultimate evolutionary explanations' leads to 'unnecessary confusion as to what constitutes a valid answer to the question of why an animal does what it does'. The biochemical analysis of 'caste-specific' pheromones in honeybees by Plettner *et al.* is a marvellous example of the interplay between 'proximate and ultimate factors'. Gadagkar concludes with an Utopian vision where evolutionary biologists become biochemists and vice versa. It would appear that there is still life in lipid biochemistry.

Revolutionizing life science education

Most scientists and educationists appear to agree that all is not well

with the state of university science education in India. Academies have pondered over the issue and there have probably been many eminently forgettable reports commissioned by successive generations of planners and governments. The National Science University proposal which aroused an extended debate in this journal, highlighted the widespread concern that science education in universities needs a dramatic reorientation. In an article on page 960, Modak details a proposal for an integrated 5-year M Sc course in life sciences. The growing importance of the biological sciences and the high hopes that have been raised by the ongoing biotechnology revolution, emphasize the importance of focussing on this area. Modak's proposal for integration of various subdisciplines will find an echo in other areas of science. Few will quarrel with the synthetic approach espoused by Modak and none will argue with the need for favouring inventiveness in students. However, a sobering thought is that we have rarely been short of good solutions on paper to important problems. The real difficulty lies in implementing any new programme, cutting through thickets of resistance to change. In a strongly worded postscript, Modak argues that his suggested experiment in education should be supported 'because it is revolutionary and original. It is a direct attempt to replant the cured soil of higher education in science with an epigenetically modified and better-yielding variety.' Deeds must follow words.

P. Balaram

Current Science

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We expect that electronic submission will result in quicker processing for publication.

CURRENT SCIENCE

Volume 71 Number 12

25 December 1996

CORRESPONDENCE

Observation on urban rhesus monkeys

Certain ecological aspects and behaviour patterns of urban rhesus monkeys (*Macaca mulatta*) that I observed as an amateur might be of interest to some of your readers and those interested in investigating related problems in nature.

For more than four years, two rhesus monkeys (1 male and 1 female) inhabited certain localities of north Calcutta and exhibited some interesting foraging patterns.

While raiding my roof-top Bonsai garden every day during two seasons each year (just prior to the monsoon and again just before winter), they fed on the fleshy inner side of exposed roots of *Salmalia*, the bark of *Terminalia arjuna*, and all parts of *Ocimum sanctum* except its roots. *Andrographis panicu-*

lata, which has a bitter taste, was also taken by them.

It is interesting to note that all these plants are medicinally important. Moreover, they also appeared to specifically consume those parts of the plants that are known to be medicinally useful. It is also noteworthy that none of the plants on which the monkeys fed died as a result of injury. They did not totally destroy any plant during any of their visits and also did not attack any particular plant on consecutive days. The fact that, in the course of 4 years, these limited resources were not exhausted is possibly significant. It should be of interest to note whether free-ranging monkeys display such sustainable exploitation of natural resources.

In course of their feeding, the monkeys also dispersed the seeds of *O. sanctum* and *A. paniculata*, thus compensating to some extent the loss that was inflicted.

Last year, the local police took away my study subjects. Soon, however, the empty niche of the rhesus was filled by human langurs (*Presbytis entellus*) who had not invaded the area during the last 4 years. In comparison with the rhesus monkeys, the langurs were far more destructive and did not exhibit any of the features of limited resource exploitation shown by the former.

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NEWS

Technological advances in the biological control of insects

Biological control, the corner stone of Integrated Pest Management (IPM), along with host-plant resistance is central to the pest management paradigm. To be more effective, it has to integrate diverse programmes involving natural enemies and microbials (bacterial, viral and fungal), besides kairomonal elements from host insects. With the advent of efficient, high quality mass rearing and delivery system and the ecofriendly, biorational techniques involved in microbial control, biological control has come to be considered as a

technology-oriented exercise. Timely production of the highest quality material supported by adequate research, development and technology transfer become very relevant to efficient biological control. Interest in these aspects has led to increased commercialization of biocontrol production highlighting the need for a healthy competition among entrepreneurs. With this background, a symposium on 'Technological advances in the biological control of insects' was organized at the Entomology Research Institute on October 11

and 12, 1996 to take stock of the existing technologies and suggest modifications where needed.

M. S. Swaminathan (MSSR Foundation), in his inaugural address, highlighted the advances and progress made in the 'Green Revolution' era in increasing the food production in India particularly during the last 30 years. Swaminathan cautioned the entomologists on the use and abuse of chemical pesticides and the current problems with high input, high tech agriculture. He desired that a blend of host-plant resis-

tance and biological control would sustain in checking the pests of crops and that a relook into the whole gamut of pest management would be rewarding in the years to come. Swaminathan also emphasized the need for integrating information technology with Integrated Pest Management (IPM) to fill the gap in spreading the IPM. He suggested that use of computers to educate and spread the knowledge among farming community must be given due consideration. This would result in group action and would educate the farmers and bio-environmental management of agroecosystems.

T. N. Ananthakrishnan (Entomology Research Institute, Loyola College, Madras) in his introductory remarks indicated that though mass rearing has become a routine technology, increased efficiency and mechanization of beneficial arthropod production are important for future success of augmentation. To be effective the natural enemies have to be retained in target areas with prospects of efficient location of hosts and as such technology for managing the environment becomes equally important. Future challenges for developing augmentation technology exist in such areas as mass rearing, quality control, storing and application. Quality control has such components as production standard, production facility and monitoring the performance in the field. Production efficiency and field performance of natural enemies depend upon diverse parameters such as survival of immatures, size, fecundity, sex ratio, longevity and host-seeking ability. Maintenance of genetic variability is another component of quality control in mass rearing involving, hybridization of field collected material with that from laboratory line. Interinstitutional co-operation is vital for the success of commercial natural enemy production as well as maintenance of ethics in the production and distribution of natural enemies.

T. M. Manjunath (Pest Control (India) Ltd. Bangalore) emphasized that with the increasing significance of biological control as the foundation of integrated pest management, there is a growing demand for biocontrol agents. The success of such programmes depends mainly on the efficient and economic mass-production, ready availability, timely application and adequate releases of quality biocontrol agents.

Recent advances made in the *in vivo* and *in vitro* rearing technology for biocontrol agents like *Trichogramma* and other parasitic wasps, *Chrysoperla*, ladybird beetles, phytoseiid mites, indicated this trend. Progress has been made in the methods of packing, storage and application techniques of natural enemies and the role of commercial insectaries and other organizations, including extension agencies, in transferring and popularizing the biocontrol technology and IPM, is commendable.

Speaking on the commercial production of biopesticides, M. Swamiappan (Tamil Nadu Agricultural University, Coimbatore) indicated that about 35 biocontrol agents comprising predators, parasitoids and insect pathogens have been found to be very promising and amenable for mass production and field use. A healthy competition among entrepreneurs is required so that biopesticide production units will be playing, an effective role in making available quality and cost-effective products for the farming community. Quality control norms are to be standardized for each and every product as they are living biocontrol agents. Streamlining is necessary in this new line of entrepreneurship by generating documents for registration, data for quality control, consideration of certain ethics and code of conduct for the production so that the real concept of biological control is perceived in order for the goals and aims to be achieved.

Highlighting the need for improved technology for mass rearing of Trichogrammatids and their factitious host *Corcyra cephalonica*, Navarajan Paul (IARI, New Delhi) opined that mass production of suitable factitious host is an important component in any biological control programme so as to ensure timely production of sufficient quantity of natural enemies for field releases. Among the factitious hosts, the Rice Meal Moth is an important factitious host that is multiplied all over the country for production of various natural enemies including Trichogrammatids. Studies indicated that the progenies of *Trichogramma chilonis* and *T. exiguum* reared on the eggs of *C. cephalonica* recorded higher fecundity and longevity as compared to those reared on *Sitotroga cerealella*. The present method of mass production of *Cor-*

cyra cephalonica is labour intensive and the labour involved in the mass production is amenable for exposure to the moth scales which are known to cause allergic reactions in many individuals. A Close Type Rearing System has been developed in which exposure of the workers to moth scales is minimized and the manual moth collection is totally eliminated. Among the rearing media used for larval rearing, maize was found to be the best, resulting in high productivity moths and better quality of the egg parasitoids. A simple cleaning device for removal of scales from *C. cephalonica* eggs was developed with a cleaning efficiency of 95%. With the use of modified oviposition cage that could be fitted below the Closed Type Rearing System, eggs could be cleaned directly without exposing the worker to scales with a cleaning efficiency of 99%. A manual operated egg card making machine has been fabricated in which *Corcyra* egg cards containing approximately 1 ml of eggs, could be prepared in six seconds. Exposure of *Corcyra* egg cards containing 0-24 h old eggs for 2 minutes to UV radiation from a 30 W lamp from a distance of 5 cm sterilized the host eggs completely, thereby eliminating the larval hatching from unparasitized eggs. A suitable illumination chamber to provide 100 lux light intensity was developed for efficient parasitoid rearing. A parasitization chamber, in which 20 ml of eggs could be exposed simultaneously for parasitization, was developed which resulted in uniform parasitization and lesser proportion of runts at 1:7 mother to daughter card ratio with an exposure period of 48 h. The economics of mass production of *T. brasiliensis* both by conventional and improved methods was worked out considering an average production of 70 ml *Corcyra* eggs per day.

D. N. Yadav (Gujarat Agricultural University, Anand) discussed the mass rearing techniques for the predators *Chrysoperla cornea* and *Mallada boninensis* developed by using eggs of rice moth *Corcyra cephalonica*. The techniques involved (1) Larval rearing in a group in a round galvanized iron sheet cage, (2) Two-phase larval rearing in multicelled plastic louvers, (3) Single phase larval rearing in Biotech Bioassay trays. The feasibility of these rearing

techniques was tested for both the species. Results revealed that larval rearing in bioassay trays and two-phase larval rearing in multicelled plastic louvers proved effective for both the species. In the case of *C. carnea*, the percentage adult recovery was maximum (90.23) when larvae were reared in biotech bioassay tray followed by two phase larval rearing in multicelled plastic louvers (75.64%) and group rearing of larvae in round galvanized iron sheet cage (55.7%). In the case of *M. boninensis* when larvae were reared in biotech bioassay tray, the adult recovery was 71.56% followed by larval rearing in multicelled plastic louver (67.09) and group rearing (13.91). The data on fecundity, longevity, genetic deterioration, egg production, storability of the predator at low temperature are given. Advantages and disadvantages were also discussed.

Referring to the role of entomopathogenic nematodes in the biological control of insects, C. V. Sivakumar (Tamil Nadu Agricultural University, Coimbatore) indicated that species of *Steinernema*, *Heterorhabditis bacteriophora* and *H. indicus* have established in Tamil Nadu, and the bacterial symbiont associated with *Steinernema* sp. closely resembles *Xenorhabdus poinarii*, while *X. luminescens* is associated with *H. indicus*. The virulence of *S. carpocapsae* and the native isolates has been established against a number of major insect pests. Plant host-induced variation in the susceptibility of *Spodoptera litura* larvae to *S. carpocapsae* exist. Virulence of *S. carpocapsae* is altered by the host insect on which it multiplies.

Foliar application of *S. carpocapsae* has been found effective against the groundnut leaf miner, *Aproaerema modicella* on groundnut and *Spodoptera litura* on sunflower. Addition of a phagostimulant to nematode spray suspension increases the effectiveness of *S. carpocapsae* against *S. litura*.

K. S. S. Nair (Director of Kerala Forest Research Institute, Peechi, Trissur) discussed the new design of a light trap, in view of biological control operation requiring more accurate monitoring of pest populations. An indigenously designed novel light trap, powered by a Solar Photovoltaic (SPV) System for use in remote areas where electricity is not available, was discussed. The light

source is a 20 V 'black-light' tube which is switched on automatically at dusk, controlled through the SPV system facilitates automatic operation for pre-set periods. The insect can be trapped into a bottle or a net-covered, walk-in chamber from where live insects can be selectively collected. Efficiency of the trap is reported on the basis of taxa of insects collected. The trap has several advantages over conventional light trap-facility to operate without electricity, operation for specific time periods, use of a black-light tube for greater effectiveness and facility for collection of live insects. A portable model with a 12 V black-light tube has also been developed and is under test.

S. Jayaraj (MSSR Foundation, Madras) highlighted the importance of Nuclear Polyhedrosis Virus (NPV) isolated from two ecosystems in Tamil Nadu, from the points of view of its pathogenicity, safety, bioefficacy, shelf-life, mass culturing techniques and field release. The virus biopesticide shows great promise in *Helicoverpa* management. Various methodologies concerning the establishment of laboratory colony of the pest, insect culturing techniques, equipments and materials needed for (a) Adult handling, (b) Egg collection and storage, (c) Egg sterilization, (d) Egg/larval handling, and (e) Pupal harvest and storage were discussed. The ingredients and methods of preparing semi-synthetic diet for the larvae were highlighted. Egg surface sterilization techniques and adult handling methods were explained, and methods of culturing NPV in the host insect and the extraction, purification and formulation of the virus also discussed. The standardization and quality control of the NPV were given much emphasis (Figure 1).

N. Ramakrishnan (Indian Agricultural Research Institute, New Delhi) discussed the growth of polyhedrosis virus in silk worm tissue culture, which provides a useful technique involved in tissue culture and difference cell lines can be developed and employed for various forms of biological research besides production of insect viruses. To date a total number of 1600 viruses have been reported, causing diseases in about 1100 species of insects and mites. About ten insect viruses (nuclear polyhedrosis viruses and granulosis viruses)

are used in large scale which are produced on the living host. The accompanying technological development in this form of production are (i) effective and cheap mass rearing media for host insect, (ii) automation in mass rearing of the host including inoculation and collection of viruses, (iii) quality control, standardization and formulation, (iv) the application technology and (v) marketing strategies. The possibilities of increasing the production of the virus by administering the host larvae with methoprene and improving the efficacy by genetic manipulations are discussed. Constraints in *in vivo* production were also explained.

R. J. Rabindra (TNAU, Coimbatore) explained that insect culture technology along with molecular techniques is currently being extensively used in developing improved viral pesticides which can give a boost to biocontrol. The successful development of baculovirus expression vector systems has given an impetus to the development of cell culture technology and consequently baculoviruses have emerged as promising ecofriendly pest management agents. Insect cells are routinely used for cloning of viruses, development of mutants and viral strains with desirable attributes like enhanced virulence and persistence. Recent advancements in molecular techniques have enabled the construction of recombinant viruses with greater pest control potential. The most crucial process of cotransfection of the cloned DNA and the wild type viral DNA takes place in insect cells grown in a suitable medium. Commercial application of the baculovirus expression vector system has opened the avenue for large-scale production of insect cells through liquid fermentation technology and *in vitro* production of viruses in insect cells. Various *in vitro* techniques have been developed for increasing cell yields and optimising virus production. Cell fusion techniques may provide hybrid cells capable of yielding greater amounts of virus.

P. Narayanasamy (Annamalai University, Annamalai Nagar) highlighted technological aspects of mycoinsecticides. Fungi, in particular, are reportedly very effective against the brown planthopper (BPH) and other pests of rice as field ecosystem is quite conducive for the fungal parasite. The fungi which were predominant included,

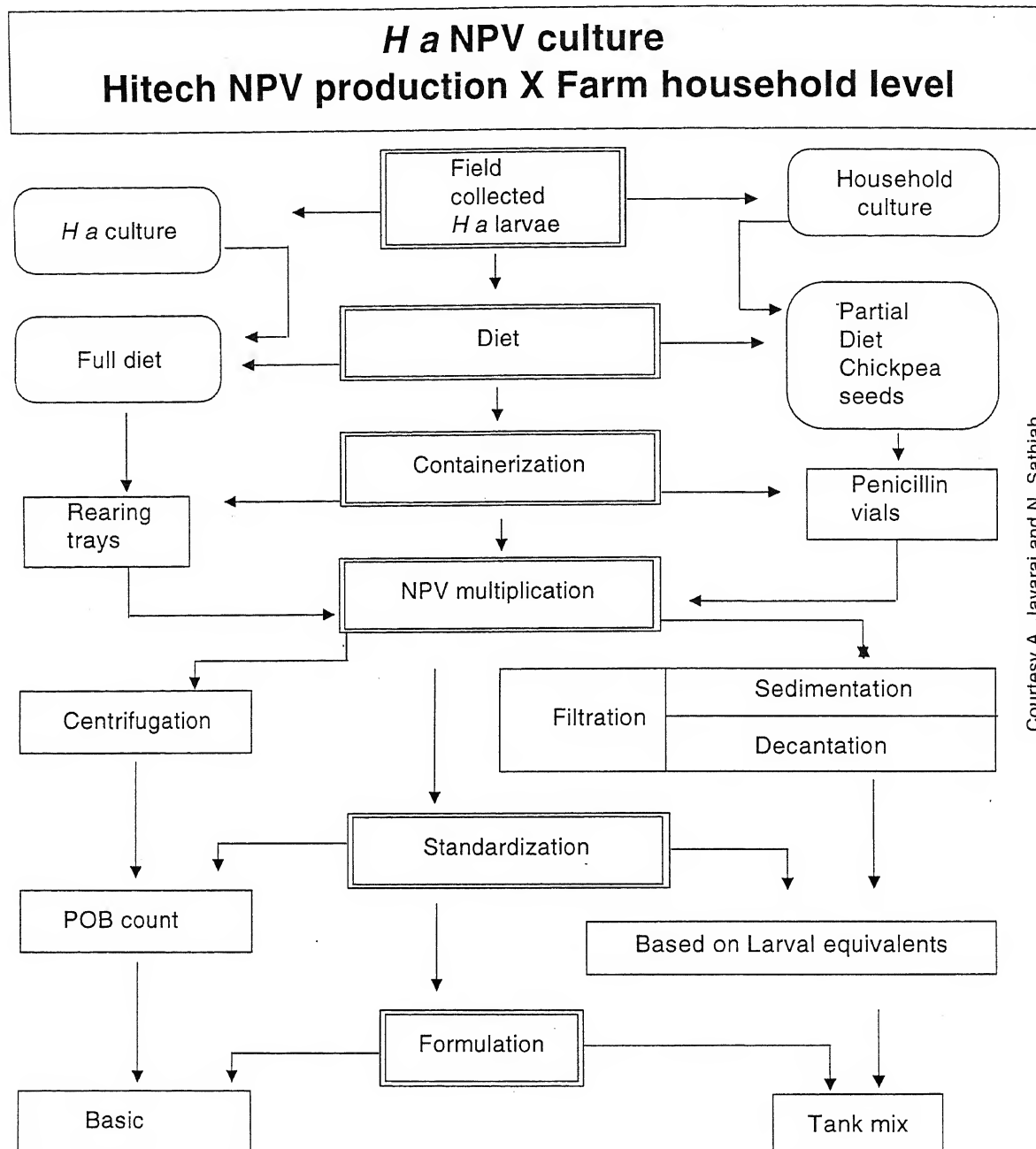


Figure 1.

Pandora delphacis, *Metarrhizium flavoviride* var. *minus*, *Beauveria bassiana*, *Zoophthora radicans*, *Nomourea rileyi* and *Paecilomyces farinosus*. The fungus *Pandora delphacis* which occurs in many districts of Tamil Nadu is a potential cure to the BPH menace. Hence the pathogen, after checking for various cultural characteristics was subjected to development as mycoinsecticide.

Two mycoinsecticide formulations such as dust (10%) and wettable powder

(70%) were prepared with broken sorghum. These formulations were evaluated under field conditions. Of these, mycoinsecticide 70% WP was found to be more potent in killing the BPH to 66.15% than other treatments tested. Moreover, efficacy of the formulation was found uniform when tested at Coimbatore, Mandya and Hyderabad. It is significant that the cost of a kilogram of the product is only Rs. 10/-, which is far less than the chemical insecticides. The potential for the use of mycoinsecticide

appears more valuable for pest problems of rice plant and hence it needs encouragement and mass use by the farming community.

S. Uthamasamy (Tamil Nadu Agricultural University, Coimbatore), coordinating the discussions, laid stress on such aspects as quality control in biological control; improvement of technology; new area of research; registration of biocontrol products/laboratories; training and implementation technology and industry/institute inter-

actions. S. Jayaraj presided over the concluding session which generated newer ideas in the area of biocontrol technology.

Quality control in biological control

Spurious materials, adulterations, substandard and poor genetic stocks are being marketed and it was suggested that there should be a network system within the All India Coordinated Research Project on Biological Control of Crop Pests of ICAR, State Agricultural Universities (SAUs), ICAR Institutes to monitor the quality of the biocontrol products marketed in our country. Further, the ICAR, Department of Biotechnology (DBT) and State Departments of Agriculture should enforce this aspect of biological control which would go a long way in improving the quality of biocontrol products.

Improvement of technologies

The urgent need for improving the existing technologies was also stressed, variations in the effectiveness of existing biocontrol products, arbitrary recommendations and very poor impact of such products on target pests are re-

ported. Hence, an earnest effort should be made to refine the technologies which would sustain the momentum gained in this area of biocontrol.

New area of research

A very large number of natural enemies are available for exploitation in the management of pests. The biocontrol potential of reduviids, anthoreids, spiders need to be studied. Further, new pathogens such as fungi, bacteria, nematodes offer scope for research and a need to be studied. Recent reports indicate that poly DNA viruses suppress the immunological reactions of the host and hence there is need for initiation of research in this area.

Registration of biocontrol products/laboratories

There is need for regulating the mass production of biocontrol agents to enable the farmers to get quality products from registered laboratories. A guideline on quality parameters, storage, transport and field release should be supplied along with the products for the benefit of users. This would educate the farmers on proper handling of these

products for effective check of the target pests.

Training and implementation technology

Farmers, involved in the production and use of biocontrol agents should be trained by institutional trainings, farm-based trainings and other innovative approaches would spread the message of biointensive approach in pest management. The communication media and computers should be utilized to the maximum extent in educating the people. Software for biological control targeting crop-based approaches *vis-à-vis* pest-based approaches would pay rich dividends.

Industry/institute interactions

An effective interaction between agro input industries and research organizations in promoting the concept of biological control would be useful, as we enter a new era of pest management in the 21st century.

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Discovery of a new effect: CFM effect

S. K. Ghosh

A report on the discovery¹ of a new effect, named Continuous Frequency Modulation Effect (CFM effect) is reported here. The CFM effect occurs in the power spectrum of oscillators undergoing a continuous modulation of the vibrational frequency. Agrawal *et al.*¹ have shown that a single sharp line, normally characteristic of an oscillatory system, broadens and exhibits a wealth of fine structure components when the system undergoes a continuous frequency modulation. Then also gives the relationship between the fine structure and the frequency modulation rate. The fine structure so observed has been termed as the 'CFM fine structure'.

For an analytical justification of the CFM effect, the authors present a detailed mathematical treatment of a pulse, $Y = A \sin \omega t$, of duration T such that ω is continuously varying with time:

$$\omega = 2\pi\nu_0 (1 - kt).$$

If the pulse duration T and the frequency modulation rate constant k are such that $2kT < 1$, then it is shown that the power spectrum of such a pulse exhibits CFM effect – the existence of a wealth of fine structure components. Further, it is shown, numerically as well as analytically, that the position of the fine structure extrema depends linearly upon the initial oscillator frequency and the square root of the absolute value of the modulation rate. The peak-to-peak spacing is shown to be proportional to the square root of the absolute value of the modulation rate.

Such CFM effect has been experimentally observed by the authors while the resonant frequency in the NMR studies

is continuously changed by varying the main magnetic field B with time. In this connection, the authors also suggest a method of identifying the electronic transients in an NMR spectrum by using the CFM effect. The authors write: 'This observation suggests a method for identification of such transients in a complex NMR spectrum. One simply removes the field lock and takes an NMR spectrum with B varying. All the NMR lines will broaden and split, but the electronic transients will be unaffected. Once identified they can be safely subtracted from the NMR spectrum.'

The CFM effect has also been verified by a computational study of different types of oscillators such as harmonic system with an exponentially modulated frequency with and without amplitude depression.

In several classical trajectory studies of chemical reactions, it has been observed in the past by different research groups that the power spectrum of a molecule transferring vibrational energy during a reaction contains unexplained fine structure. Agrawal *et al.* point out that with the help of the CFM effect not only is the fine structure explainable, but one can also deduce the rate of energy transfer by the molecule from the knowledge of the CFM fine structure.

Such a point has been presented in detail by correlating the rate of vibrational energy transfer with the rate of frequency modulation rate and the computed CFM fine structure for different diatomic molecules vibrating under the influence of the Morse potential.

For the application of the CFM effect to the molecular reaction dynamics, the

authors discuss three examples: (1) Determination of vibrational relaxation rate coefficient from the knowledge of the CFM fine structure in a computational study of HONO in a cryogenic rare gas matrix, (2) The use of CFM effect to extract the rate of energy transfer from a diatomic molecule to a surrounding matrix cage of Ar atoms, (3) The instantaneous energy transfer rate from the knowledge of the CFM fine structure. However, the CFM effect may not be applicable in the molecular systems if the vibrational frequencies cannot be continuously modulated due to the constraints imposed by quantum mechanics.

Since the CFM effect is applicable whenever there is a continuous frequency modulation of a vibrating system, it becomes an effect of fundamental importance and its application cannot be limited to a few cases. It may be of importance in Physics, Chemistry, Electronic-engineering, Astrophysics, and many such disciplines where the vibrational frequencies are continuously modulated even for a short duration of time. Further, from the point of view of research in mathematics, the CFM effect widens the scope of research in the Fourier transform of different types of functions representing vibrations with continuous frequency modulation.

1. Agrawal, P. M., Sorescu, D. C., Kay, R. D., Thompson, D. L., Raff, L. M., Conrey, J. B. and Jameson, A. K., *J. Chem. Phys.*, 1996, **105**, 2086–2700.

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Research Snippets (compiled by A. V. Sankaran)

The parting of Indo-Australian plate

In the past two decades, scientists have been noticing certain abnormalities in the lithosphere south of India which forms part of the northward drifting

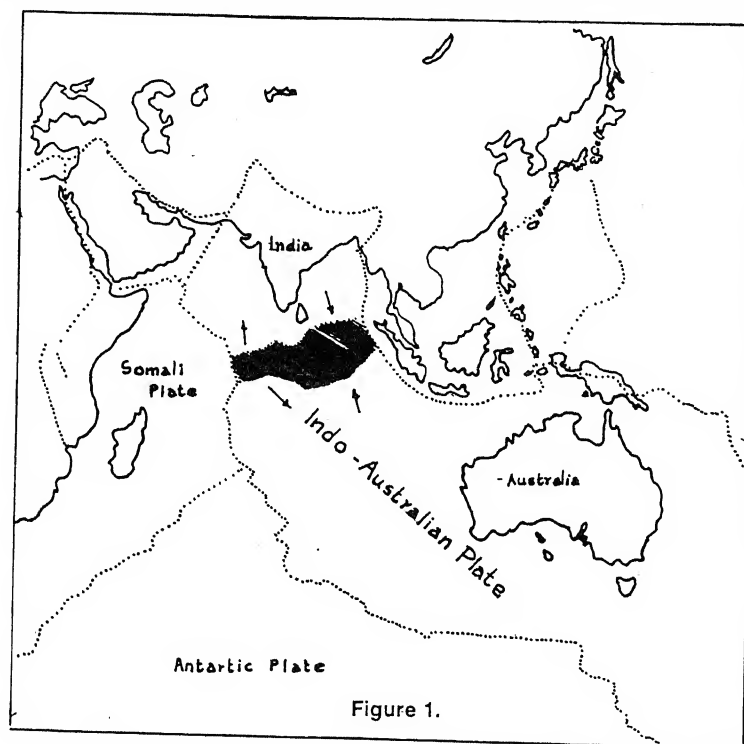


Figure 1.

Indo-Australian plate. This area, which is almost in the central portions of this plate, was exhibiting signs of deformation or crumpling – a feature normally associated with tectonic plate edges only (Figure 1). A team of workers – James R. Cochran (now in MIT), J. K. Weissel of Lamont-Doherty Earth Observatory and Florence Jestin of Ecole Normale Supérieure (Paris) investigated data¹ gathered during oceanographic expeditions in 1986 and 1991 and from a study of faults in the ocean bottom, they have calculated that India and Australia are currently moving in different directions and hence must be sitting on separate plates. They have even calculated that the deformed lithosphere is nearly 900 km across – wider than the borders between other tectonic plates and that the split was formed during the last 8 million years. If confirmed, the new studies would bring the number of major plates to 17.

1. Lamont-Doherty, *Sci. News*, 1995, **148**, 123.

Archaeopteryx – No more oldest known bird

Some new finds of avian fossils from Liaoning Province (NE China) and nearby Korea dating back to the Jurassic have shed fresh light on trends in bird evolution. These fossil finds described¹ by Chinese and US palaeontologists, are believed to pre-date *Archaeopteryx*, the oldest bird fossil discovered in 1861 from shales in Bavaria (Germany). The new fossil bird, about the size of a pigeon, named *Confuciusornis sanctus* has features which are a blend of modern and Jurassic times. Its toothless, horny beak, feather-covered legs which are short above and long below the

knee – features that were thought to have developed in birds only during Cretaceous and later times – make this Chinese fossil find closer to modern birds. But the design of the forelegs and long recurved claws on the feet suggestive of its arboreal habit are more allied to *Archaeopteryx*. These discoveries are considered to be very exciting inasmuch as they have questioned existing views of *Archaeopteryx* ancestry to modern birds and have pointed to possibilities for other side branches to the avian tree of which *Archaeopteryx* branch, perhaps, is one, but which apparently met

with a dead end. These finds lead to an obvious conclusion that if birds were already diverse in Jurassic, the still more primitive of the species must have taken to wings as early as late Triassic – some 60 million years before *Archaeopteryx* flapped in the Jurassic skies.

1. Lian-Hai Hou, Zhonghe Zhou, Martin, L. D. and Feduccia, A., *Nature*, 1995, **377**, 616.

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Yellow fever threat?

In the wake of dengue and dengue hemorrhagic fever (DHF) epidemic in Delhi and neighbouring states, there is a heightened concern among the public health experts of the possible outbreak of yellow fever in the Indian sub-continent. This lurking fear emerges from the global experience in the emergence and re-emergence of at least 30 infectious diseases¹. Yellow fever virus belongs to flavivirus group, and other members of this group have already entered India and gradually assumed epidemic proportions as exemplified by the appearance of DHF in Calcutta in 1963 and Japanese encephalitis (JE) in North Arcot Dist., Tamil Nadu in 1958. These diseases moved from east towards west, and have now occupied vast territories throughout India, thus raising an alarm of the possible future scenario of communicable diseases and exposing the public health preparedness and monitoring capabilities. Other examples are the re-emergence of once-decimated diseases like kala-azar and malaria, the recent epidemic of plague, and emergence of drug-resistant parasites, vector resistance, and ecological succession of vectors with better adaptations. Tropical climate and environmental degradation makes India a hot bed of new infections and, therefore, warning signals for yellow fever are already there. Vector control which was so successful in the control of communicable diseases has unfortunately taken a back seat. The entomological component in most public health programmes in the endemic countries is the weakest and often non-existent, whereas vector control still remains the most effective and rational approach to vector-borne disease con-

trol. Endemic countries will do well by giving entomology its due place in the public health programmes. What makes India vulnerable to yellow fever are an unprecedented increase in *Aedes aegypti* populations due to urbanization, industrialization, water storage practices, rural water supply and population migration. *Aedes aegypti* 'type form' and var. 'queenslandensis' are sympatric² and both are potential vectors as demonstrated by transmission studies³. Similarly *Ae. albopictus* is susceptible to yellow fever virus⁴. *Ae. aegypti*, breeds in containers, rain water collections and invades new territories through used tyres and solid waste dumps and finally diffuses through piped water supply and then proliferates, whereas *Ae. albopictus* is sylvatic and native to India. This mosquito invades peripheral wooded areas and gardens in urban areas. Indian are susceptible to yellow fever as they lack antibodies⁵. Virus invasion is a possibility through the large volume of traffic from yellow fever endemic countries in Africa and South America. Although yellow fever has not moved to any other continent, there is a geographical spread from west coast to east coast of Africa, south of Sahara. There is, therefore, a possibility that large-scale population movement, ecological upheavals, population explosion and developments for better living may break these barriers. Furthermore, quarantine health checks and special sanitation measures introduced under the International Health Regulations⁵ to prevent yellow fever entry through air and sea ports into India are not functional. Once the virus enters India either through infected humans or the vector, it can spread through

the local vectors. Indian monkeys, viz. *Macaca mulatta mulatta* and *Macaca radiata* are susceptible to yellow fever virus and *Ae. albopictus* can maintain a zoonotic cycle and become a lasting source of infection. To prevent this from happening, quarantine health checks and *Aedes* control measures should be applied rigidly and introduced at all ports of entry in the Indian sub-continent. A surveillance system should be developed to monitor vector populations and the antibody profile in the indigenous populations. Municipal and building bye laws should be adapted and implemented uniformly throughout the country to control vector breeding on sustainable basis. Solid waste disposal should be managed professionally. Emergency strategic planning and adequate stocks of vaccines and drugs should be at hand to fight the accidental entry of new diseases such as the yellow fever.

1. Anonymous, *Down to Earth*, 1996, 5, 60.
2. Kalra, N. L., *Bull. Indian Soc. Mal. Com. Dis.*, 1968, 5, 307-334.
3. Sinton, J. A., *Health Bull. no. 22*, GOI Press, Delhi, 1938.
4. Mitchell, C. J. et al., *J. Am. Mosq. Contr. Assoc.*, 1987, 3, 460-465.
5. WHO, *International Health Regulations*, 1969 (ed. Third Annotated), WHO, Geneva, 1983.

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Occurrence of a 'fossil' crocodilian vertebra from Hassan District, Karnataka State – A preliminary report

The fossils were recovered accidentally during levelling operation by a bulldozer prior to the agricultural activities in Mosale Hosahalli village, Hassan

Taluk and District (Figure 1). This was brought to our notice by one of our students of the same area who also helped us in collecting the samples.

From our observation about the material, it is unlikely that the material was originally in the present place and that it was found *in situ*. It might have been

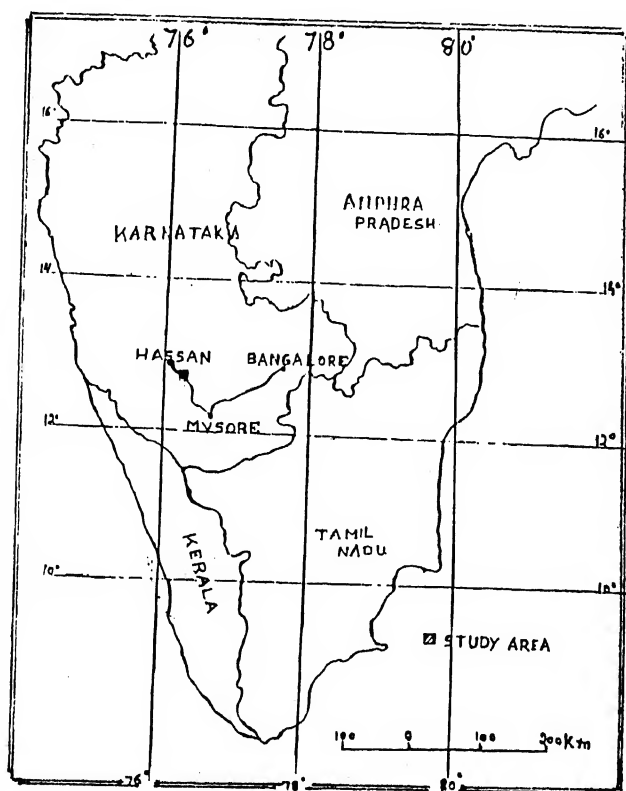


Figure 1. Location map of Mosale Hosahalli.

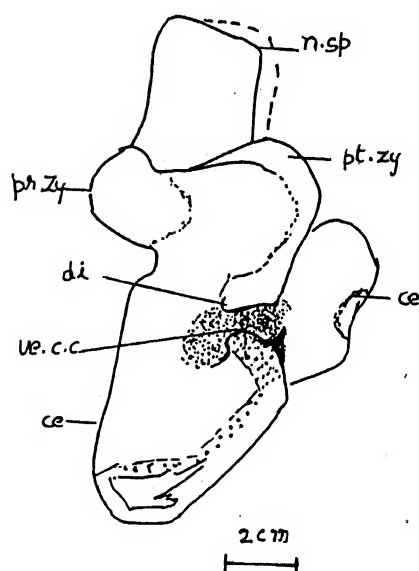


Figure 2. Left lateral view of vertebra. ce, centrum; di, diapophysis; n. sp, neural spine; pr. zy, pre zygapophysis; pt.zy, post zygapophysis; ve.c.c., vertebrocostal canal.

carried by water currents to the place from where it was collected.

The country rock of the area is peninsular gneiss enclosing schistose rocks of

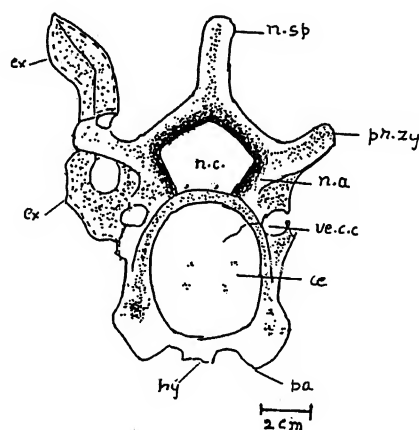


Figure 3. Anterior view of vertebra. ce, centrum; ex, extraneous part; hy, hypapophysis; n.a., neural arch; n.c., neural canal; n.sp., neural spine; pa, parapophysis; pr.zy, pre zygapophysis; ve.c.c., vertebrocostal canal.

Bababudan group, Dharwar Supergroup spanning an age of 2600 to 3000 m.y. in the Archean¹. These rocks are highly metamorphosed and are commonly devoid of any fossil remains^{1,2}. This brief geological account covers a general

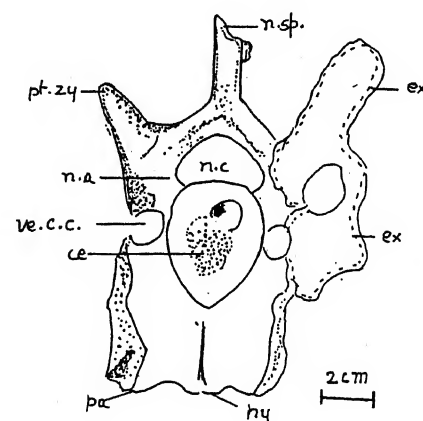


Figure 4. Posterior view of vertebra. ce, centrum; ex, extraneous part; hy, hypapophysis; n.a., neural arch; n.c., neural canal; n.sp, neural spine; pa, parapophysis; pt.zy, post zygapophysis; ve.c.c., vertebrocostal canal.



Figure 5. Left lateral view of vertebra.

study of the entire Bababudan group as a whole which also includes Mosale Hosahalli from where the collection of our material was made. The soil of the area is grey in colour and contains clay and mica particles. Kankar nodules are abundant below this mantle of soil.

The vertebra measures a length of 9 cm, height of 14 cm and width (in the region of zygapophyses) of 9 cm. The material is cement grey in colour and weighs about 670 g, which is quite heavy when compared to the modern crocodilian vertebra, which is pale yellow in colour and relatively lighter also.

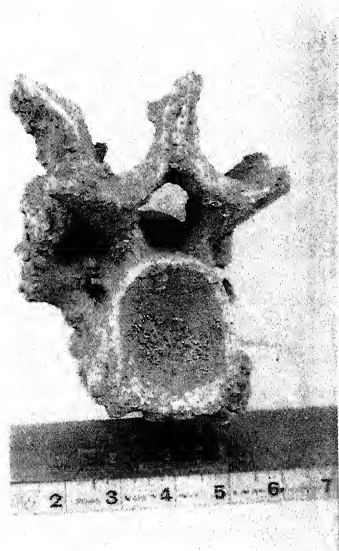


Figure 6. Anterior view of vertebra.



Figure 7. Posterior view of vertebra.

The vertebra shows the centrum, neural arch and accessory structures. The centrum is procoelous, being broad and low anteriorly and narrow and high posteriorly. Dorsolaterally it bears a pair of vertebrocostal canals for the passage vertebral arteries. The diapophyses have been partly preserved. Feeble traces of hypapophysis and parapophyses are also seen.

The neural spine arises as a dorsal median vertical ridge from the neural

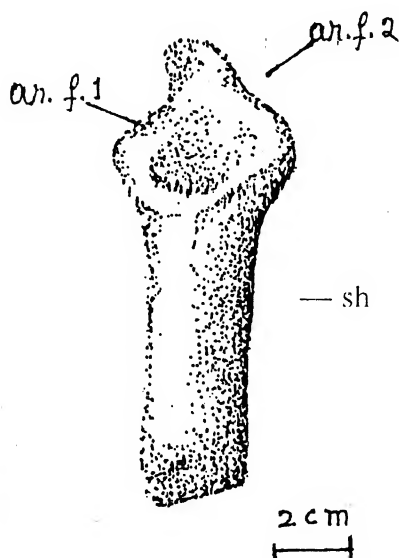


Figure 8. Dorsal view of fibula. ar.f.1, facet for articulation with astragalus; ar.f.2, facet for articulation with calcaneum; sh., shaft.

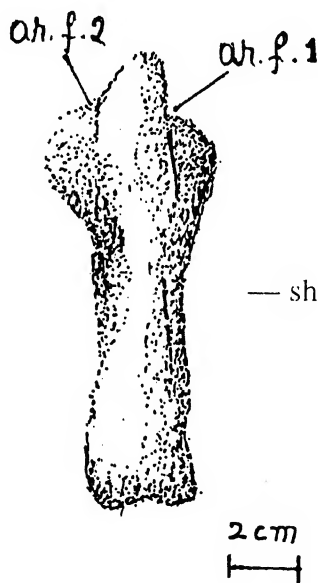


Figure 9. Ventral view of fibula. ar.f.1, facet for articulation with astragalus; ar.f.2, facet for articulation with calcaneum; sh, shaft.

arch. The zygapophyses extend as lateral flattened horizontal processes from the sides of the neural arch. The pre- and post-zygapophysis have broad and shallow articulating facets³ (Figures 2-7).



Figure 10. Dorsal view of fibula.

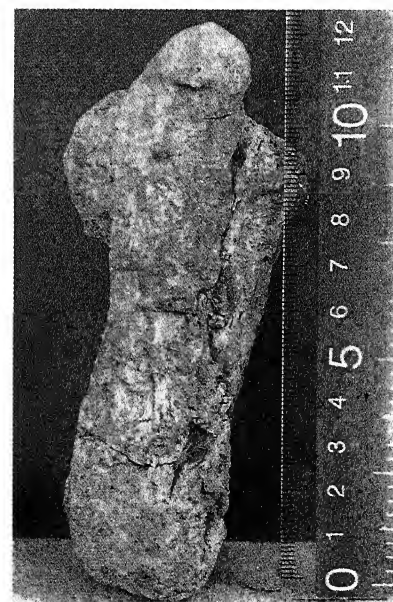


Figure 11. Ventral view of fibula.

In view of the above details regarding the fossil vertebra, the same has been identified as a cervical vertebra of the order, Crocodilia. However, there is an extraneous part attached to the zygapophyses on the right side of the vertebra, whose identity and presence are not clear.

From the above description, it is clear that our material is distinctly different from the corresponding modern crocodilian vertebra.

In the fibula the length of the shaft measures 12 cm and its diameter is 3 cm. The articulating surface of the distal end has a diameter of 5.5 cm. The material is cement grey in colour and weighs 250 g which is quite heavy when compared to the corresponding fragment of modern crocodilian fibula.

The fossil bone shows a thick shaft and a terminal expanded portion showing a central shallow depression and articulating surfaces. It also shows a conical process posteriorly.

The above remains are probably the distal part of fibula of the order Crocodilia. Probably the proximal two-thirds of the bone is wanting⁴.

The fibula articulates with the tarsus at its distal end. In the living crocodile, there are two proximal tarsal elements articulating with tibia and fibula. These are the astragalus and calcaneum. Fibula articulates with the astragalus at its inner articulating surface and with the calcaneum at its outer articulating surface at the region of bony projection (Figures 8–11).

From the study of the crocodilian 'fossil' vertebra and fibula, it seems that a very small area of sedimentary fos-

siliferous rocks is lying over the non-fossiliferous schistose rocks which cover most of the area.

This is the first report of the occurrence of crocodilian 'fossil' skeletal remains from this place which incidentally gets the name Mosale Hosahalli. The term Mosale in Kannada means a crocodile. Perhaps, because of occurrence of crocodiles in this place in the distant past in a large body of water, probably a river, the village might have obtained its current name, Mosale Hosahalli.

There are reports of similar 'fossils' being found in the late Pleistocene gravel beds of peninsular Indian rivers (Ashok Sahni, pers. commun. 1995).

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Agnostid trilobites from the Cambrian Sequence of Zanskar and their stratigraphic significance

The Zanskar area forms a vast mountainous region between the great Himalayan range in the south-west and Indus Valley in the north-east and occupies the southern part of Zanskar region lying between lat. 30°00–33°08'N and long. 77°15' to 77°25'E (Figure 1). Srikantia *et al.*¹ have given a detailed geological map of the Zanskar region. Gaetani *et al.*² also discussed the significant stratigraphy and sedimentology of the Zanskar. Dungrakoti *et al.*³ also recorded trilobites from Zanskar Valley and assigned an upper Cambrian–Ordovician age. Whittington⁴ described some trilobites from Kurgiakh section of the area. Nanda and Singh⁵ worked on the sedimentology¹ of the area. Since Zanskar Basin shows continuity with the Spiti Basin, the lithostratigraphic nomenclature adopted here

is identical to the one adopted for Spiti Basin. A complete succession of Palaeozoic–Mesozoic rocks is exposed along the right bank of Zanskar river in the Lingti valley (Figure 1). Rocks of Cambrian to Permian age exposed in the Valley are rich in fossils. The sequence in the southwestern part of Zanskar is represented by Giambal, Haimanta and Kanawar groups. In Kurgiakh valley the rock formations found at the base belong to the Batal Formation and Kunzam La Formation of Haimanta Group, consisting of greyish or greenish micaceous schists which attain a huge thickness. The upper Kunzam La Formation according to Hayden⁶ corresponds to the Parahio 'series' of Spiti from where Middle Cambrian trilobites have been recorded. The Haimanta

group of the Zanskar is overlain by the Kanawar Group which is divided into three formations, namely Lipak, Po and Ganmachidam formations. Kanawar Group directly overlaps the Thango Formation in most of the Zanskar Basin.

The fossiliferous Cambrian sequence in this valley has a faulted contact with Gumbranj Granite. A diversified trilobite fauna was collected from this sequence. The fauna signifies a Middle Cambrian age. The lowermost bed of this sequence consists of a laminated micaceous shale chiefly attaining a great thickness and is devoid of fossils. Gaetani *et al.*² grouped this shale under Phe Formation of Precambrian to Early Cambrian age.

The trilobite fauna obtained from this area include the following taxa.

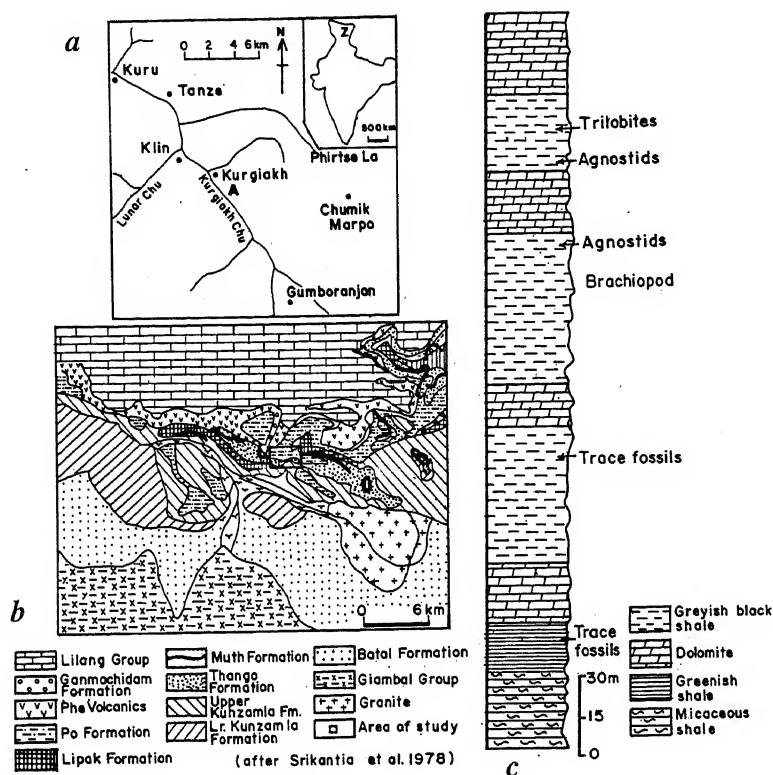


Figure 1. a, Location map of Zaskar area showing Kurgiakh section; b, Geological map showing the area studied (After Srikantia *et al.*¹); c, Stratigraphic column.

Repository. The fossils have been deposited in the Palaeontology Museum, Geology Department, Jammu University. Fossil specimen numbers are indicated for each taxa after the figure number in the explanation to Figure 2.

Acadagnostus scutalis, Salter, 1886 (Figure 2: 1, 2, 5).

The specimens correspond with *Acadagnostus scutalis* in its evenly curved cephalon and pygidium and well preserved axial pygidial node. The specimens differ from *Hypagnostus cf. clipcus* in the absence of subquadrate pygidium, short axis and width being greater than length.

Peronopsis tramitis, Opik, 1979 (Figure 2: 8, 9, 10, 21).

The specimens resemble *Peronopsis tramitis* in a number of morphological characters such as subcircular cephalon, small and circular frontal glabellar

lobe, well-preserved pygidial axial node. They differ from *Peronopsis rakuroensis* Kobayashi in the shape of the cephalon and strong median glabellar node.

Peronopsis cf. longinqua Opik, 1979 (Figure 2: 7, 11, 17).

The specimens differ from *P. prolixa* in the shape of the posterolateral spines. They also differ from *P. (Itagnostus) elkedraensis* in the shape of the glabella and presence of small node on the anterior glabellar lobe. The form is known to have three lobes in the axial region which is not clear in the present specimens and hence they are tentatively referred to this species on the basis of subrounded cephalon, presence of prominent pygidial axial node and its placement.

Peronopsis (Itagnostus) cf. elkedraensis (Etheridge Jr.) Opik, 1979 (Figure 2: 14, 16, 19).

The specimens are comparable with *P. (Itagnostus) elkedraensis* because of the angularity at the anterolateral cephalic margins, absence of preglabellar furrow, position of the pygidial node and subrounded shape of the pygidial marginal furrow. The specimens differ from *P. montis* Mathew in the presence of the glabellar node and absence of posterolateral angularity of the pygidial rim.

Peronopsis sp. (Figure 2: 22, 24, 25).

The specimens contain a number of pygidial and cephalic characters of that of the genus *Peronopsis*, such as nearly circular cephalon with parallel acrolobes, glabella divided by a transglabellar furrow, in the ratio of 2:3, pygidium semicircular to rectangular shaped, pygidial axis gently narrowing posteriorly, etc. Because of their poor preservation they have been identified only up to generic level.

Peronopsis amplexa Robison, 1982 (Figure 2: 3, 4, 6, 13).

The specimens correspond to *P. amplexa* in the cephalic and pygidial characters especially in their smaller average size, wide axial lobes, weak segmentation in the pygidial axis and in the absence of marginal spines. Robison⁷ has discussed in detail the comparative morphology of *P. amplexa*.

Diplagnostus floralis Opik, 1979 (Figure 2: 18, 23).

The specimens compare with *Diplagnostus floralis* on the basis of wide pygidial axis at the anterior side and abruptly pointed rear side, rapid widening of the marginal furrow and two faintly-preserved furrows dividing the pygidium into three lobes.

Doryagnostus magister Whitehouse, 1936 (Figure 2: 12, 15, 20).

The specimens resemble *Doryagnostus magister* in a number of physical characters such as dimensions of cephalon nearly equal to the pygidium, width of the pygidium which is more than its length, curved nature of pygidial spines, etc. The specimens also show resemblance with *Doryagnostus incertus* but can be differentiated on the basis of the curved nature of marginal spines. The specimens can also be differentiated from *D. natalibrae* in the absence of scrabicles over the surface of the acrolobes and bow-shaped transglabellar furrow.

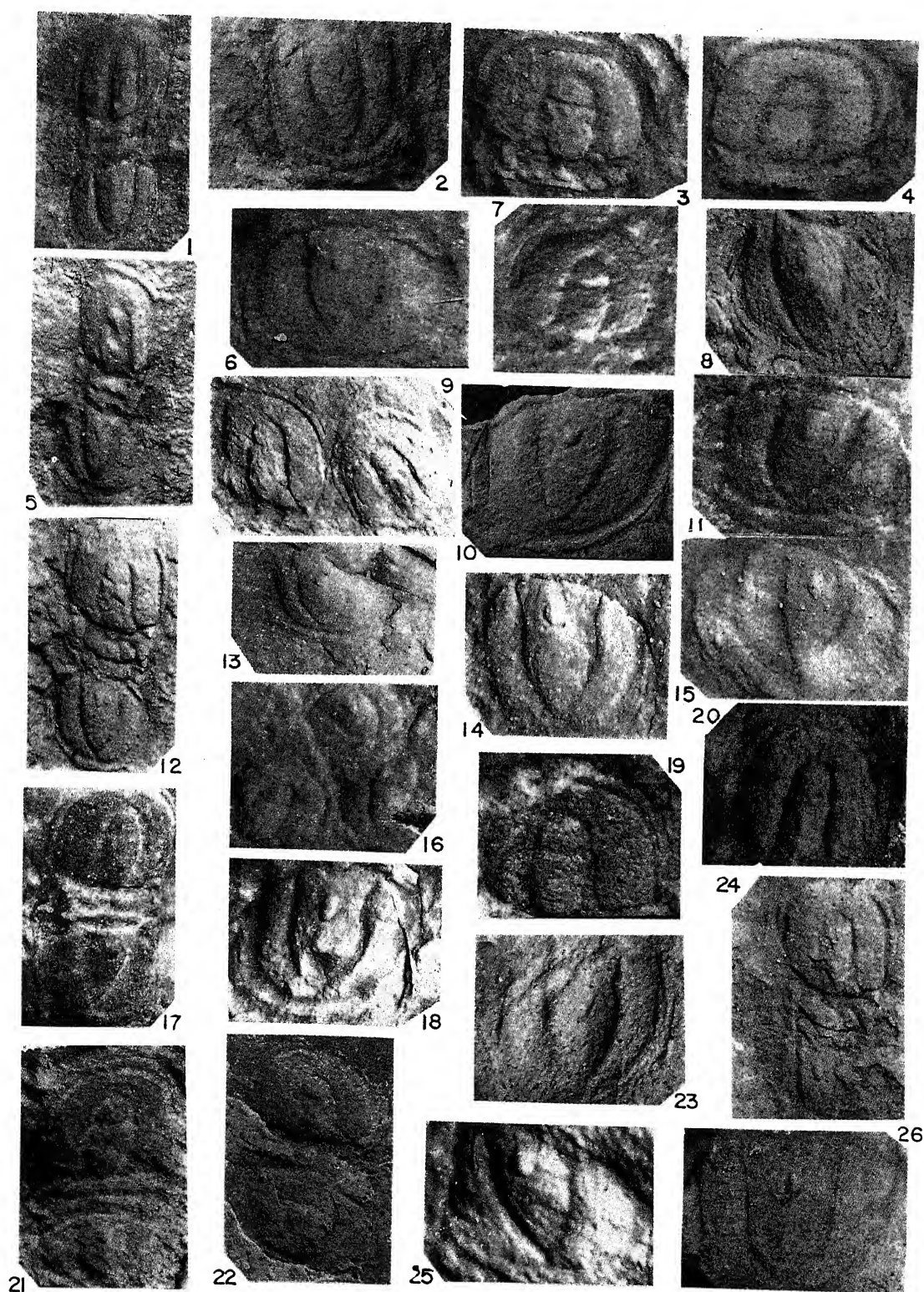


Figure 2. *Acadagnostus scutalis*: 1 \times 8.4 (KUF-706), 2 \times 9.7 (KUF-704), 5 \times 9.7 (KUF-720). *Peronopsis tramitis*: 8 \times 10.1 (KUF-702), 9 \times 8.1 (KUF-714), 10 \times 8.3 (KUF-721), 21 \times 12.8 (KUF-706). *Peronopsis cf. longinqua*: 7 \times 5.5 (KUF-705), 11 \times 13.6 (KUF-723), 17 \times 10.5 (KUF-469). *P. (Itagnostus) cf. elkedraensis*: 14 \times 7.4 (KUF-707), 16 \times 11.2 (KUF-699), 19 \times 15.0 (KUF-716), 26 \times 10.8 (KUF-728). *Peronopsis* sp.: 22 \times 11.1 (KUF-714), 24 \times 12.1 (KUF-705), 25 \times 14.5 (KUF-718). *P. amplaxa*: 3 \times 11.7 (KUF-724), 4 \times 9.3 (KUF-705), 6 \times 11.7 (KUF-703), 13 \times 8.4 (KUF-728). *Diplagnostus floralis*: 18 \times 23.5 (KUF-723), 23 \times 10.3 (KUF-728). *Doryagnostus magister*: 12 \times 18.2 (KUF-710), 15 \times 13.8 (KUF-716), 20 \times 10.4 (KUF-708). The specimen numbers are given in parenthesis.

SCIENTIFIC CORRESPONDENCE

The agnostid fauna from the Middle Cambrian of Zaskar is closely comparable with that of the adjoining basins of Kashmir and Spiti. In Kashmir the agnostids consisting of different species have been described from *Solenopleura-Tonkinella* zone of Middle Cambrian⁸. Agnostids from Zaskar are also comparable with the assemblage recorded from *Ptychognostus gibbus* zone of Middle Cambrian in Australia⁹ and with the same zone in North America¹⁰. The Agnostid fauna presently described and other polymerid fauna indicate a shallow marine condition of deposition.

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Gas emanations and subterranean sounds/microearthquakes in Marathwada, Maharashtra, India

After the great devastating earthquake of Killari on 30 September 1993, the Marathwada region is again experiencing microearthquake activity. This microearthquake activity is also accompanied by subterranean sounds and gas emanations. Earthquakes are many times accompanied by gas eruptions at high pressure. When this gas at high pressure comes out from small pores or fractures, it makes sharp sound which includes rushing sound¹. The Killari region of Latur district has received two tremors on 29 August 1996, at 12.10 am and 12.30 am and two tremors on 2 September 1996, at 1.30 am and 1.52 am. These tremors were of magnitude 4 (*Lokmat* newspaper dated 29 September, 1996). These tremors were followed by gas emanations at Poharegaon 29 km NW of Latur and at Gunj 35 km NW of Nanded (Figure 1). Subterranean sounds were also heard in Poharegaon.

A week following the earthquake tremors received on 29 August 1996, there is report of gas emanations and it continued for a few days. At Poharegaon, gas emanations started on 6 September 1996 and continued till 12 September 1996 while at Gunj emanations started on 7 September 1996, and continued till 9 September 1996. The description of the sites is as follows:

Site 1 Poharegaon (Taluka Renapur, District Latur): The site of gas emanation is about 2 km NW of Poharegaon.

Gas emanations were first felt by animals and they turned their way because of the hissing sound. Then villagers reported an odourless emission coming out up to 1 ft height from small pores of

0.5 cm diameter in an area of about 0.5 m diameter. The pores were observed only on the upper 4 inches of soil cover (Figure 2 a). The hissing sound could be heard up to the distance of 125 feet. After closing one hole, a

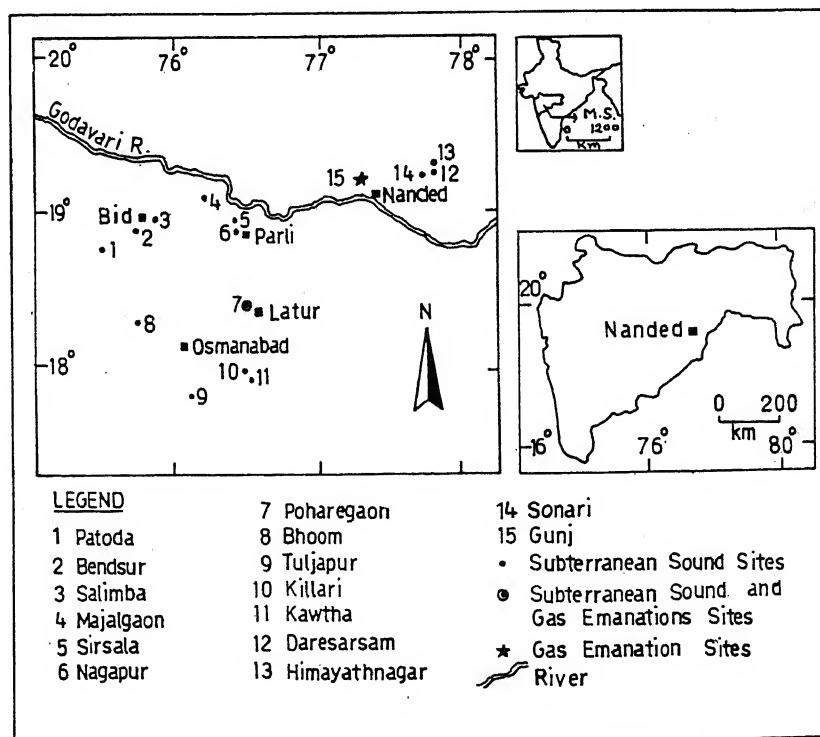


Figure 1. Location map of the sites.

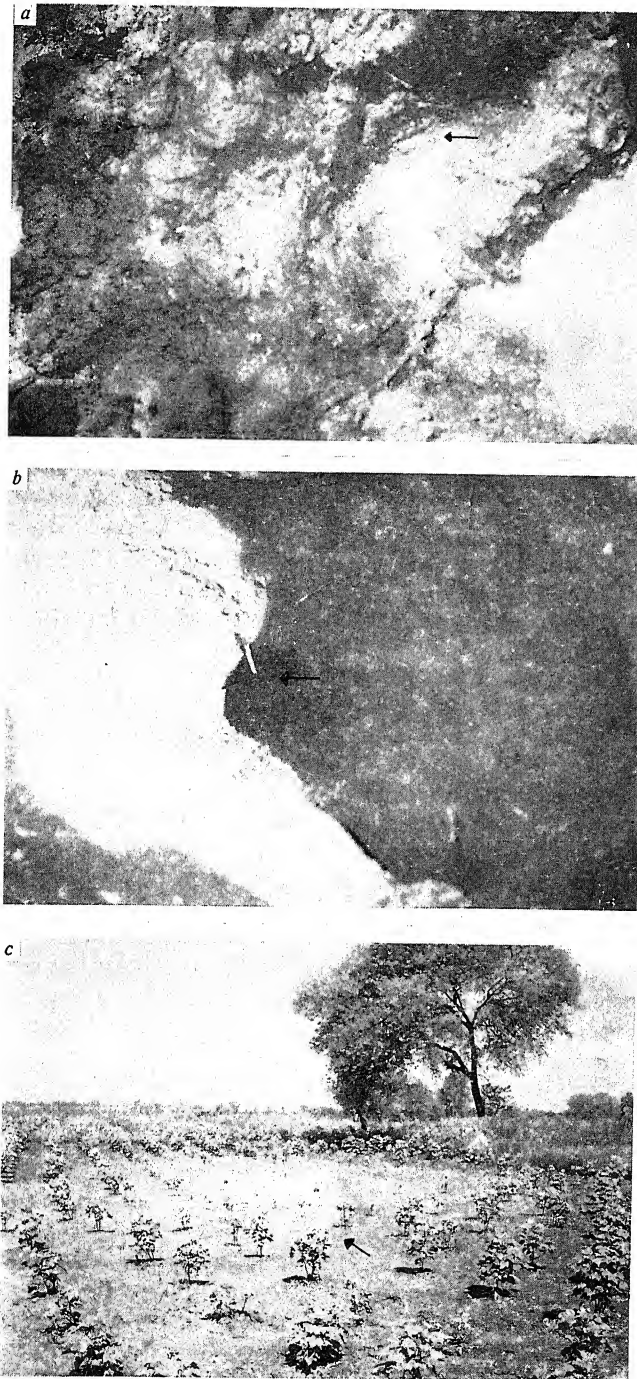


Figure 2. *a*, Gas bubbling out (Poharegaon); *b*, Small hole through which gas blew out (Gunj). Note that villagers have excavated the soil up to 2 ft to find out the source of gas emanation; *c*, Water clogging near the site of gas emanation (Gunj).

new one used to open up and after pouring water on these holes, water showers were thrown into air up to the height of 1/2 ft. Subterranean sounds are also reported.

The gas emanations occurred after the region had received heavy rains. After the earthquake of 30 September 1993 also,

gas emanations had occurred just after the Latur region received heavy rains².

When our team visited the site, water clogging was observed just by the side of the gas emanation site, and gas emanations had almost subsided. Gas was seen to bubble out only when water was poured on these holes. Termites were

reported to be coming out during emanation. The temperature recorded in the holes from which gas was coming out appeared to be normal. It was 27.5°C and there is no report of ground heating during gas emanations.

Site 2 Gunj (Taluka Vasmat, District Parbhani): The site of gas emanation is about 1 km south of Gunj. The river Ahana which follows the lineament trending N60W–S60E is on one side of the gas emanation site and a small stream which also follows a minor lineament trending E–W is on the other side of the gas emanation site. There is no effect of microearthquake in this village but gas emanations are reported. Subterranean sounds are not reported from this village. The gas is reported to be odourless and temperature was also normal. The area is not reported to have become hot. Gas emanation was only through one hole of diameter 1 cm (Figure 2*b*). Water clogging was observed just by the side of the gas emanation site (Figure 2*c*). Termites were reported to be coming out during emanation. Though the gas is reported to be odourless, it is reported that a person who came in contact with the gas had swelling on his hand and face and felt irritation and a burning sensation. The site could not be visited during gas emanation and hence no samples of gas were collected.

Site 1: Poharegaon area is covered by basaltic lava flows. A basaltic flow consist of two units – a lower massive and compact basalt and upper vesicular or amygdaloidal zeolitic basalt. The massive basalt is dense, dark grey to black, fine grained with only few large vesicles. Amygdaloidal basalt is brown to greenish with almost all vesicles filled with secondary mineral zeolites³.

Amygdaloidal basalt occurs towards SE side of the gas emanation site and compact basalt towards NW side. Gas emanation had occurred at the contact of these two rock types. Amygdaloidal basalt is slightly tuffaceous and highly weathered and fractured and can be broken into pieces even after applying a little pressure by fingers. The soil thickness over this rock type is about 25 ft. This rock obviously shows more porosity and is a good potential zone for groundwater. Dug wells present in this rock have ample amount of water even during summer. A vertical borehole of 50 ft depth taken in a dug well showed

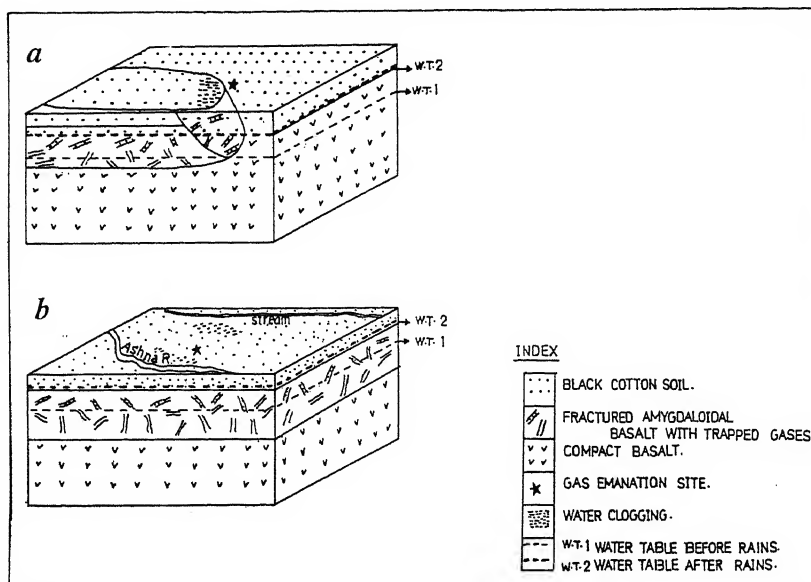


Figure 3. Explanatory diagram for the gas emanations sites (not to the scale).

fast rate of drilling and yielded good amount of water. No rock particles came out of the bore and all the material was lost in the cavities or fractures present in the highly-weathered basalt. Soil cover over compact basalt is about 60 ft. Borewell of 140 ft depth taken in the adjacent rock did not yield water (Figure 3 a).

Site 2: Gunj area is also covered by basaltic lava flows⁴. A basaltic flow consists of two units – a lower massive and compact basalt and an upper vesicular or amygdaloidal tuffaceous basalt. The massive basalt is dense, dark grey to black, fine grained. Amygdaloidal basalt is pinkish to reddish and contains brown volcanic glass⁴. At the site of gas emanations, soil thickness is about 35 ft, below which weathered and fractured amygdaloidal basalt is present. Below amygdaloidal basalt, compact basalt is present which is devoid of fractures. Groundwater occurrence is reported only from the weathered amygdaloidal basalt which is acting as a reservoir rock (Figure 3 b).

Sites of subterranean sounds/microearthquakes and ground cracks which are noticed in Marathwada region for about a month now include Daresarsam, Bhoom, Salimba, Killari and Latur which were also reported to have suffered earlier from these types of activities in 1993 (ref. 2).

The following is the description of the sites where subterranean sounds/cracks are reported in Marathwada re-

gion for the last one month (Source: Daily newspaper *Lokmat*, *Samana*, National TV, News and Civic authorities).

1. Patoda, Bid district: From 10 to 17 September 1996 explosion-like sounds were heard (Source: Good Morning India National TV News, 18 September 1996).

2. Bendsur, Bid district: From 21 to 23 September 1996, explosion-like sounds were heard. A ground crack at the foothills was also reported.

3. Salimba and Majalgaon, Bid district: From the last few days, subterranean sounds were heard.

4. Sirsala, Bid district: A ground crack of 20 ft in length was reported from this village.

5. Nayagaon, Bid district: A ground crack 150 ft in length and 1 ft in width is reported.

6. Nagapur, Parli taluka, Bid district: From 21 to 24 September, subterranean rumbling sounds were heard.

7. Van, Parli taluka, Bid district: For the last few days subterranean sounds have been reported. Dam wall crack of about 1 km in length is reported to have a width of 1 inch and depth of about 2½ feet. Chandrashekhar, Senior Geologist, GSDA has visited the place.

8. Poharegaon, Renapur taluka, Latur district: On 28 and 29 September 1996, rumbling sounds are reported from this village. It is reported that sounds travelled from east to west. A small tremor is also felt.

9. Latur: On 14 September 1996, fire cracking sound from a well was reported to be coming out. The well was dry during summer. After heavy rains it received water and was followed by subterranean sounds.

10. Bhoom, Osmanabad district: On 21 September 1996, thundering sounds were reported, and after the sounds some houses received minor cracks and 10–12 small pits developed in this region.

11. Tuljapur, Osmanabad district: On 21 September 1996, loud subterranean sounds were heard which were followed by shattering of tin sheds and small shaking was also felt. Abnormal behaviour of pet animals was also reported.

12. Killari, Latur district: On 28 September 1996, cloud thundering-like sound was heard which travelled from east to west. A tremor was felt for 4 seconds and shattering of sheds (tin and asbestos) was reported. Similarly, adjoining villages Gangapur and Ausa in Latur district experienced a small tremor. This tremor has caused cracks and peeling of plasters from the rehabilitated houses in Killari Tanda.

13. Kawtha, Umarga taluka, Latur district: On 17 and 18 September 1996, subterranean sound was reported to be coming out from the ground nearer to the site where a 2 × 2 feet depression developed after the tremor.

14. Sonari, Himayatnagar and Daresarsam, Nanded district: From 14 to 17 September 1996, explosion-like subterranean sounds were reported. After the sounds, shattering of sheds, peeling of plaster from walls and falling down of utensils from shelves were reported.

Marathwada region is experiencing microearthquake activity, accompanied by gas emanations and subterranean sounds. Earlier also there were reports of subterranean sounds and gas/steam emanations and microearthquake activity about two months prior to 30 September 1993 earthquake⁵. Gas emanations have been reported just after the earthquake on 29 August 1996 and 2 September 1996 after the region received heavy rains. Amygdaloidal basalt in this region is highly weathered and fractured and fast drilling rate and loss of material in borewell itself suggests presence of cavities and is probably the site for trapped gases. Heavy rain has resulted in the water table rising and

saturation of the region with groundwater. This might have enhanced the gas eruption. The earthquake tremors received recently might have opened up the fractures and in all probability gas emanations are due to release of trapped gases in amygdaloidal basalt as a result of a microearthquake. In both the cases of gas emanation, no fractures were observed on the surface. There is probably a change in fracture porosity of the weathered and fractured basalt flows present at deeper or shallow levels caused by the tremor shaking⁶. Gases were coming out from small pores. Termites were also reported to be coming out during emanation and both the sites were small mounds in nature. The rise in water table might have displaced the entrapped air that would find its way up through termite burrow holes which have acted as gas outlets. It is to be noted here that near the gas emanation sites, water clogging is observed. In the absence of radon and

helium analysis, it is difficult to link these gas emanations to deep origin and it is preliminarily guessed that gas emanations at these sites are related to escape of trapped air in fractured basalt from a shallow level. The burning sensation experienced by the person might be explained by the release of injurious gas from the decay of biomass which is used in the field as fertilizers.

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Uranium mineralization in the Palnad sub-basin, Cuddapah Basin, Andhra Pradesh, India

Recent investigations by Atomic Minerals Division have brought into focus the presence of uranium mineralization hosted in fracture zones in granite close to the unconformity with the overlying Srisailem quartzite in Lambapur area^{1,2}, in the north-eastern part of the Cuddapah basin. Investigations further east of the Srisailem sub-basin have revealed the presence of significant concentration of uranium (up to 0.55% U_3O_8 with negligible ThO_2) in the quartzite grouped under Banganapalle Formation of Kurnool Group. This radioactive quartzite is exposed in the western parts of the Palnad sub-basin near Koppunuru, Alugurajupalle and Dwarakapuri villages (Survey of India Toposheet No. 56 P/7; 16°24'0"N; 79°20'20"E), Guntur district, Andhra Pradesh. Petrographically, the host rock is quartz arenite (orthoquartzite) with a high degree of mineralogical and textural maturity. Preliminary field data indicates that the mineralization has been influenced by major faults/fractures which facilitated the migration of (hydrothermal?) mineralizing fluids.

The main uranium-bearing minerals in this rock are pitchblende, coffinite, phosphouranylite and metazeunerite associated with sulphides of copper, lead and iron. An attempt is made in this note to bring out the salient features of this new uranium find in the Palnad sub-basin, which has

enhanced the uranium potentiality of the northern parts of the Cuddapah basin.

The Banganapalle quartzite is the oldest lithounit of the Palnad sub-basin (equivalent to Kurnool sub-basin) in the north-eastern corner of the crescent-shaped Cuddapah basin³. In this part of

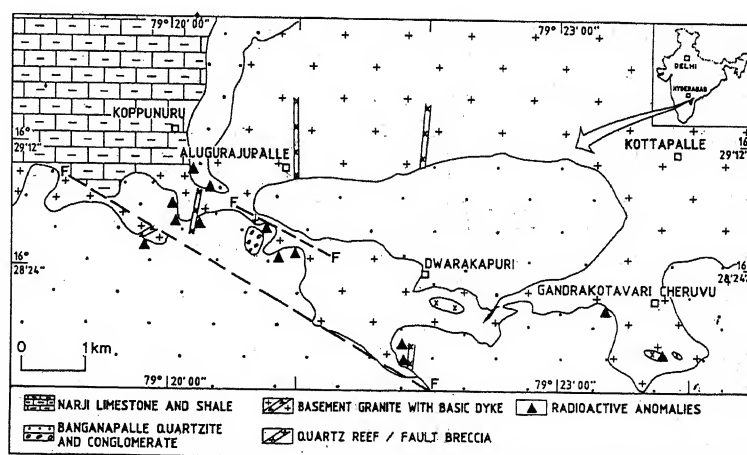


Figure 1. Geological map of Koppunuru–Dwarakapuri area.

SCIENTIFIC CORRESPONDENCE

the Cuddapah basin, the Banganapalle quartzite unconformably overlies the basement granite (Figure 1). The basal unit of this formation is a conglomerate/pebbly quartzite, followed by ferruginous and grey quartzite exhibiting primary sedimentary structures such as ripple marks and current bedding. These are invariably capped by pitted quartzite. At places, this quartzite has intercalations of black shale. Euhedral pyrite and specks of chalcopyrite occur throughout this sequence. The quartzite is subhorizontal with a low dip of 2° to 6° towards southeast. Major WNW-ESE trending faults, besides NNE-SSW trending minor faults, are the major structural features of this area and are marked by fault breccia, fault scarp and quartz reefs.

The preliminary petrographic studies on the samples suggest that the host rock is quartz arenite (orthoquartzite), which is well sorted, with subrounded to rounded, fine to medium-sized, grains of monocrystalline quartz, cemented by authigenic silica. In a few samples, lithic fragments of granite-mylonite, glauconite, chlorite, opaques and carbonaceous matter are present (8–20% modal). The rock fragments of crushed granite indicate granitic provenance.

The presence of glauconite, with traces of carbonaceous matter, indicate marginal marine, mildly alkaline and reducing environment of deposition. The high degree of mineralogical and textural maturity and the chemical data of major oxides (analysed by rapid method of analysis using spectrophotometry, atomic absorption spectrometry, volumetry and flame emission photometry techniques) (Table 1) point to a stable erosional cycle of granitic provenance.

Uranium mineralization has been recorded in the grey and ferruginous quartzite of Banganapalle Formation and the quartz reef in basement granite. Mineralization occurs in several patches (Koppunuru – 20 m × 25 m to 150 m × 20 m, Alugurajupalle – 200 m × 1–5 (thickness), Dwarakapuri West – 300 m × 5 m (thickness) to 100 m × 2 m (thickness) and Dwarakapuri South – 400 m × 100 m) in a roughly E–W trending linear belt of 8 km, bounded on the southern side by a major fault (Figure 1). Uranium concentration, in the quartzite ranges from 0.013 to 0.55% U₃O₈ (Table 2) and the maximum concentration is observed at Dwarakapuri. Preliminary petrographic and X-ray diffraction studies indicate that the uranium mineralization is mainly at-

tributed to the primary uranium minerals – pitchblende (UO₂ · UO₃) and coffinite (U(SiO₄)_{1-x}(OH)_{4x}), the secondary uranium minerals – uranophane (Ca(UO₂)₂(SiO₃OH)₂ · 5H₂O), phosphouranylite (Ca(UO₂)₃(PO₄)₂(OH)₂ · 6H₂O) and metazeunerite (Cu(UO₂)₂(AsO₄)₂ · 8H₂O) and the accessory uranium minerals – chevkinite (Ca, Ce)₄(Fe Mg)₂(Ti, Fe)₃Si₄O₂₂) and zircon (ZrSiO₄). These are associated with chalcopyrite (CuFeS₂), pyrite (FeS₂) and galena (PbS). The uranium-bearing minerals occur along fractures, cavities and bedding planes. Some amount of uranium is also adsorbed on goethite, glauconite, clays, goethitized pyrite, carbonaceous matter and malachite. Interestingly the quartz reef, with associated chalcopyrite and pyrite analysed up to 0.036% U₃O₈.

The radiometric assay values indicate that most of the samples show disequilibrium in favour of parent (uranium). The disequilibrium ratio, on an average, is 1:1.35. This high disequilibrium in favour of uranium is apparently related to the high concentration of secondary uranium minerals in these quartzites.

In view of the favourable geological setting, similar to that of Lambapur^{1,2} the presence of high concentration of

Table 1. Chemical analysis of Banganapalle quartzite Koppunuru–Dwarakapuri area, Guntur Dist., A.P.

Locality	S. No.	SiO ₂	Al ₂ O ₃	CaO	MgO	FeO	Fe ₂ O ₃	MnO	Na ₂ O	K ₂ O	TiO ₂	P ₂ O ₅
Koppunuru East	1	99.00	0.25	<0.02	<0.02	0.28	0.06	0.0023	<0.10	<0.10	<0.05	0.02
	2	98.95	0.45	<0.02	<0.02	0.28	0.13	0.0036	<0.10	<0.10	0.09	0.04
Koppunuru South-east	3	98.00	0.36	0.031	<0.02	0.44	0.86	0.0015	<0.10	<0.10	0.05	0.06
	4	97.85	0.64	0.13	0.026	0.32	0.17	0.0088	<0.10	0.10	0.10	0.15
Alugurajupalle	5	88.60	1.15	0.036	0.026	0.46	9.50	0.0012	<0.10	<0.10	0.09	0.27
	6	80.60	2.87	0.062	0.040	0.36	13.04	0.0036	<0.10	<0.10	0.09	0.35
Dwarakapuri South	7	92.00	2.23	1.29	0.073	0.28	0.51	0.0033	<0.10	0.61	0.05	1.13
	8	98.00	0.55	<0.02	<0.02	0.22	0.21	0.0020	<0.10	0.22	<0.05	<0.02

Table 2. Radiometric assay data – Koppunuru–Dwarakapuri area, Guntur Dist., AP

Area	No. of samples (n)	Radiometric assay				%ThO ₂	Disequilibrium factor (%U ₃ O ₈)
		%U ₃ O ₈	%U ₃ O ₈	(Beta)			
				(Gamma)	(Gamma)		
		Range	Average	Range	Average		(Beta) (%U ₃ O ₈)
Koppunuru	8	0.014–0.077	0.039	0.013–0.083	0.039	<0.005	1:1
Koppunuru east	11	0.010–0.092	0.047	0.020–0.16	0.071	<0.005	1:1.51
Koppunuru south east	6	0.016–0.28	0.095	0.019–0.42	0.143	<0.005	1:1.50
Alugurajupalle	5	0.011–0.039	0.024	0.016–0.045	0.030	<0.005	1:1.25
Dwarakapuri	7	0.054–0.34	0.153	0.059–0.55	0.231	<0.005	1:1.51

SCIENTIFIC CORRESPONDENCE

uranium in association with sulphides in the quartzite, the discovery of uraniferous anomalies in Koppunuru-Dwarakapuri area have opened-up the entire Palnad sub-basin as a very favourable target for uranium exploration.

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MEETINGS/SYMPOSIA/SEMINARS

National Symposium on Natural Resources Management System

Date: 26-28 February 1997

Place: Tiruchirapalli

Themes include: Water-surface/ground, land use patterns, Biodiversity - Flora and Fauna - diversity, endemism, endangered species, conservation, aquaculture augmentation strategies, Human resources - Indigenous knowledge, ethnobotany, Forest and Wasteland management, Socio-political issues in conservation, Toxicology - radiation, biocides, xenobiotics, Marine bioresources, Geography, mineral resources, remote sensing and GIS, Biotechnology and resource management, Sustainable development of natural resources.

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IUFRO Symposium on Innovations in Forest Tree Seed Science & Nursery Technology

Date: 22-25 November 1997

Place: Raipur

Topics include: Seed Biology: Seed aging, physiology, biochemistry and molecular biology, anhydrous seed biology, seed improvement, synseeds, seed hygiene, seed products, quality, testing and certification, technology of seed banks, quarantine. Nursery technology and management, quality saplings.

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Science education in Indian universities: Proposal for a 5-year integrated M Sc course in life science

Sohan P. Modak

The undergraduate and postgraduate science degree courses in India lack a conceptual basis and systems approach for designing syllabi. Existing syllabi are repetitive and wasteful. An integrated 5-year M Sc in life sciences is proposed. Life sciences decipher mechanisms and processes underlying structural and functional organization and the basis of instability of the living state. These should be taught in a synthetic sequence beginning with Physics, Chemistry and with English, Computing, Mathematics and Statistics as languages during the first 3 years. The final two years should cover subject of specialization and advanced streams with a research base and management skills. Summer training and remedial courses should be obligatory.

THOUGH India is the tenth largest world economy with the third largest trained work-force in science and technology, it is still a developing country. The size of our economy primarily reflects the developments in the agriculture and the size of our population, and only secondarily the size of our industry that mostly caters to the local needs. We do not really design, develop and produce modern amenities maintaining high quality standards using our trained work-force. Barring specific cases like rockets, satellites, or a small software industry, our industrial missions involve outdated ckd kit assemblage or copying through reverse engineering. Post-war Japan, on the other hand, with practically no natural resources, started out from scratch, but where is it now? Mountain ice is the only natural resource of Switzerland¹ and yet it produces the best of everything from textiles to paper, machine tools to industrial plants, watches to microelectronics, marine engines to solar cars, etc. Its agriculture can hardly feed 60% of its population, yet the food processing giant, Nestlé, is Swiss. For a 41,293 sq km landmass and a population of about 6.8 million, Switzerland boasts of one of the largest pharmaceutical industry with CIBA-Geigy-Sandoz combine and Hoffmann-La Roche. In contrast, India has immense natural resources, practically a mosaic of all climates and biodiversities, but do we have any impact on the global trade and economy? Nation building and technological leadership require trained work-force with creative and innovative mind sets, hard work and entrepreneurship. This is what the education system should catalyse. Therefore, it is necessary to assess our educational system and its output.

The NCERT and the State Board of Higher Education devise and regulate the teaching syllabi and method-

ologies up to the 10+2 level. NCERT has maintained the tempo for a continuous evolution of syllabi to ensure a broad-based training. In contrast, an excessive specialization in college and university, without emphasizing the basic tenets of science education has led to the production of a work-force loaded with information but without integration. This situation is similar to our copying data bases with neither relevant questions to ask nor the tools to find answers. In Maharashtra, mathematics has been de-emphasized *de facto* for +2 level students opting for the biology stream. The 10+2 acts as a screen to select the 91%+ students seeking admission to medicine, engineering and technology courses. Earlier, it was respectable to study sciences and a good proportion of students around $80 \pm 5\%$ entered the science discipline. With the proliferation of private engineering and medical colleges, the screen for open seats has moved down drastically. Similarly, with the advent of professional courses such as MBA attracting better informed and versatile students, only those from the lower percentile are available for traditional M Sc. Ten years ago, admission to physics and electronics courses at the University of Pune ended at 75% marks, while today this is down to 50%. In some subjects moreover, over 60% seats have remained vacant at FY B Sc and the enrolment to M Sc will continue to decrease. Since 10 years there has been a diminishing interest in Sciences world wide and we are just beginning to see the fall-out in India. A major proportion of Indian M Sc degree holders become teachers for lack of better opportunities. Among them, less than 7% students pass the UGC-CSIR NET because of the poor quality of B Sc and M Sc training. In India we have over 200 universities and 10,000 colleges whose job is to produce trained personnel. The present education is neither learner-friendly nor user-friendly, nor has it kept pace with modern trends in sciences. Besides, most bright students joining Medicine

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and Engineering, do not go in for research and development. Let us examine all components of our science education.

To implement undergraduate and postgraduate syllabi, institutions and government provide the infrastructure. Let us examine their performance. The Boards of Studies continually dilute syllabi, ignore modern teaching methodologies, and promote obsolete and often-plagiarized teaching texts. Course structure and teaching sequence are haphazard with futile attempts to introduce 'catchy' titles under the garb of vocational courses which the teachers are not competent to teach. The laboratory exercises are obsolete, unimaginative and lack the inductive stimulus. Most postgraduate departments follow this trend. A few try to undo the damage by packaging so much material that it is difficult to digest. Either situation leads to a perpetuation of mediocrity, absence of work culture, suppression of dissent, kills curiosity, and wastes student's time, thereby preventing development of scientific temper. Boards of Studies also promote mediocre and repetitive Ph D theses supervised by guides of questionable competence and assessed by referees of equally questionable integrity. Managements are responsible for this lack of infrastructure and operate the educational institutions as money-spinners through capitation fees and forced donations. Elected Governments are the main culprits in giving higher education the lowest priority. Furthermore, many teachers participate in a parallel 'black' educational network, thriving on private coaching classes and tuitions while disregarding their professional obligations in the classroom and laboratory. Governments engage only in the rhetoric of a mass higher education and not higher education *per se*. The UGC, the body for regulating the quality of higher education, is toothless. Attempts to make passing in NET/SET compulsory for teacher appointments have failed under the pressure of various teachers' unions. All of this has led to a monumental 'academic fraud' perpetrated on the Indian student and the nation.

By mid-80s it became clear that a dramatic fix was necessary to make a quantum improvement in the teaching infrastructure and syllabi in modern biology. The National BioTechnology Board mooted the idea of introducing a training programme in M Sc biotechnology with UGC's support. For this course, science graduates were selected by an entrance test. The programme, originally restricted to 6 universities, quickly became a model for quality training in Life Sciences as it included many innovations, flexibility in its course-design and the openings for creative teaching. The programme attracted good students in the 80 ± 5 percentile. However, the geopolitical considerations led DBT and UGC to extend this programme to over 19 institutions. Consequently, the percentiles dropped below 75%, and the syllabus- and teacher-quality plummeted. Ever-changing policies on reservations for teaching posts and

lack of concept-basis have drastically reduced the effectiveness of biotechnology training programmes. For example, in Pune 3 out of 6 teaching posts still remain unfilled. This is also true of other subjects which suits state exchequers who naturally save on already depleted funding for higher education. Almost everywhere in India, a new class of education empire-builders have come to the fore who have done very little to evolve a good educational system. I agree with Sitaramam² that the Biotechnology students do well because they are basically talented and not necessarily due to skilled teachers, good syllabi on job-potential. In mid-eighties, UGC and CSIR introduced the National Eligibility Test (NET) for postgraduate research fellowships and evolved advanced syllabi hoping that this would force major revisions in the undergraduate and postgraduate syllabi in the country. I was involved in the establishment and the syllabus-framing of both Biotechnology Training programme and the NET, but, in retrospect, I find that the impact of these syllabi on teaching life sciences in India is at best disappointing. Towards the end of 80s, various surveys revealed that, excepting for a few late-bloomers, most students performing around 80 percentile at +2 level do well in B Sc, M Sc and UGC-CSIR-NET. This has direct bearing on the learning potential of the student, communication skill of teachers, the quality of syllabi and infrastructure at the +3 level. As most students opt for undergraduate science subjects in the absence of other channels, the concern for a good teacher-quality has become important.

The teacher

A teacher is the primary medium of information-transfer who also catalyses the process of learning or integrating the information into knowledge. I examine here the requirements for making a good teacher. A good teacher should be motivated and able to communicate and induce the student. The teacher should be an expert who continually updates her/his knowledge. An ideal teacher should be highly skilled and involved in the process of enquiry and research. Such a teacher would have self-respect and the mind-set with scientific temper without loss of empathy. The teacher should know that the information is not knowledge and that creative teaching involves inductive inputs, not dictations, to promote learning. A good teacher also knows that the science is introvert. It superposes philosophy, and the process of creative learning involves analysis, synthesis, dissent, high-level curiosity, and the search for truth at any cost. These are the conditions for developing the scientific temper. In contrast, the technology is extrovert requiring skills and aptitude for execution, management, innovation and enterprise. On a ten-point assessment scale, I predict that the top 2% undergraduate teachers will

score 7 or more points, the next 5% five points and the remaining 3 or less. A horizontal scale-amplification with excellent, very good, good, fair and poor, will hardly change this situation. Using the same scale, postgraduate teachers in universities may perform only marginally better. Yet, student's examination scores are high because of manipulation of the examination system. This is the epitaph to undergraduate and postgraduate teaching in India and is the reason for a low-quality output of scientific work-force. Improving teacher-quality is not a make-shift operation possible through refresher courses and monetary incentives. It has to begin in the schools and colleges. Those who have not learnt, and therefore are not knowledgeable, just should not be in this profession. Learning is solving puzzles where a picture emerges using the memory base, recall, and integration in real-time by recognizing patterns through trial and error, multiple choice selection, common sense, general knowledge, intuition, creativity and enterprise.

The student

Then, what about a potentially good student? A good student should be able to read, write, speak and listen in the languages of science and able to observe, recognize shapes, patterns and relationships between these through recall and retrieval from the memory base. A good student should have acquired skills to analyse and synthesize, and be logical in thinking to question beliefs, falsities as well as truths. The student should be on constant lookout for what is happening around with fearlessness and hard work, should have manual skills to match the information base, appreciation of quality and quantity and the desire to overcome. Unfazed curiosity is the hallmark of a good student and the cumulative experience hones the sense of intuition and leads to creativity.

The goal of education is to facilitate an individual's passage to become an ideal student. He/she should be induced to develop a correct mind-set and personality conducive to the conduct of a scientific enquiry. 10+2 syllabi do not promote language skills, the mind-hand connection, fearlessness, general knowledge, appreciation of diversity and quantity, or thinking. At this point, the student is in a formative period, with the left side of the brain still untapped. Only serious attempts to promote each ideal trait will elicit a variable but positive response of the heterozygous *Homo sapiens* that cannot be achieved through a 'mutant' selection or despotic social engineering.

University of Pune – A case study

The University of Pune administers undergraduate and postgraduate education including professional degrees in engineering and medicine in three districts in Maharashtra, namely, Pune, Ahmednagar and Nasik cover-

ing over 200 colleges. The Boards of Studies (BOS) in each subject frame the undergraduate and postgraduate syllabi. Each BOS contains 6 members elected from among Heads of College Departments teaching TY B Sc, the Head of the University department, two teachers co-opted from among teachers who are not department-heads, and two outside experts. Thus the BOS primarily represents the constituency of undergraduate teachers and frames even postgraduate syllabi. Only after syllabus-ratification by the Academic Council, heavily representative of college teachers and managements, can the course be taught.

During 1980–1984, the University placed greater trust in teachers by allowing them to allot 40% marks by internal assessment for each course, and the entire examination of the so-called C component (vocational courses) and departmental courses. Unfortunately, this led to an excessive marking (never below 90%) in internal assessment and C component-courses very much due to peer-pressure and teacher's goofing-off, as compared to an average of 50% in centralized external examinations. The syllabus framing also included preparing question banks from which at least 50% of questions were included in the external examination. In most subjects the question banks were so small the teachers restricted their lectures to these, thereby diluting the syllabus and destroying the work-culture among students. Under the pretext of syllabus modernization, some of the important topics, taught in B Sc in 60s and 70s, were not covered till later in M Sc. The responsibility for this failed experiment lies squarely on the shoulders of the 'untrustworthy' teachers. This forced the University to eliminate question banks and the C component, and to change the marking ratio for external:internal from 60:40 to 80:20. The departmental courses still serve as fodder for teachers involved in excessive marking to cover-up their own deficiencies which is hardly different elsewhere.

In mid-80s, as the admissions to University PG courses, based on merit list at the +3 level did not reflect in the student performance in MA and M Sc, two solutions were used in science departments by preparing merit lists from marks secured in the external examinations and giving academic autonomy to University departments recipient of UGC–DSA, CAS and COSIST. Such departments framed their own syllabi and conducted entrance examinations independent of college postgraduate centres. In 1985–1986, NBTB (DST)–UGC awarded M Sc Biotechnology Training Programme to the Zoology department. Earlier, TIFR had initiated a collaborative teaching programme with the Physics department, while NCL did the same with the Chemistry department. UGC recommended that all departments with DSA, CAS or COSIST should have academic autonomy and admission through a nationally advertised entrance test. While admissions to Biotechnology and

Zoology continued through entrance tests, the latter with 25% seats to students with B Sc in other science subjects, the entrance tests in Chemistry and Physics were restricted to the collaborative programmes. As the number of PG centres in Zoology increased, the total number of seats for M Sc Zoology quadrupled. Admission to University department being in part on scores in the entrance tests, good but poor students in the rural areas became attracted towards the University department that does not charge capitation fee. Only in 1996 was the botany syllabus revised to match the standards of UGC-CSIR-NET, and an entrance test introduced. However, even this trend is not prevalent in most other universities. Unfortunately, the academic autonomy is now under fire from the University legislative bodies presumably due to the loss of control over University departments. Concomitantly, admissions to life science subjects at undergraduate level have dropped as the State Board of Higher Secondary Education separated biology and physical-mathematical science streams at +2. This drastically reduced the number of postgraduate centres with spurious teaching and examination record. Other than CAS-Sanskrit, UGC also identified statistics, sociology, and mathematics departments for special assistance. But these did not opt for an entrance test as the available seats remain unfilled using B Sc merit lists. In conclusion, the faculty performance in teaching and research should allow identification of expertise in the thrust areas in different subjects. Such an exercise in botany restricted the teaching to 5 specializations out of a plethora of eight.

Teaching life sciences in India

Life science subjects are taught at the undergraduate level in a macro to micro sequence beginning with, say, the animal kingdom, then the taxonomy, speciation, study of types, morphology, anatomy and histology. Cell biology, genetics and physiological chemistry are taught in the second and third years and the same of the first year is repeated, presumably at a higher level, without paying adequate attention to chemistry, physics, biochemistry and molecular biology. The pattern is repeated in M Sc, with minimal inputs in modern biology. This sequence inculcates an analytical frame of mind, destroys the ability to synthesize, to formulate concepts, designs, structures and to understand the structure-function relationships. The lack of a synthetic sequence also suppresses innovativeness, creativity and enterprise. This is true of all undergraduate and postgraduate courses in life sciences in India taught using substandard teaching methods which condemn students to the dungeon of boredom. Life science teaching has, thus, become sterile. An occasional flash of brilliance is a fluke due either to the inherent capacity of the student, or a

rare teacher, or both acting in harmony. Life sciences, though, offer a fertile field for research in chemistry, mathematics and physics, but in India, there is no dialogue among the teachers in these subjects. Thus, we will remain as customers for information from the West.

The correct approach

Life sciences decipher the mechanisms and processes underlying structural and functional organization of the living, and the basis of instability of components and systems, entities or beings, and their associations at molecular, cellular, and organismic levels. The principle of instability is evident in atomic nuclei and particles, atoms and their isotopes. At the molecular level, weak and strong interactions affect the stability of bonds. In contrast, most macromolecular aggregates hold together through weak interactions. These aggregates combine to form cell organelles and perform structure- and composition-specific functions segregated in space (position) and time. Cell organelles perform specific function and, in desired formulations, give rise to cells. Cell communities either share all functions, or exhibit complementarity through acquisition of specialized functions, and form organs like root, stem, leaves, flowers, fruit brain, heart, gut, bones, skin, etc. A perfect blend of structurally segregated but functionally linked cell communities give rise to multicellular beings that grow or reproduce, adapt to changes in the environment, defend against toxins and parasitic attack, and exhibit societal interactions. Only this sequence ensures assimilation of the paradigm relevant to the functionality and the division of labour. Thus, a student must begin learning in this 'synthetic' sequence for generating complexity out of simplicity which will have a dramatically different effect on the mind-set and temper as it follows an architect's and builder's 'psyche' based on constituents, composition and management. It favours inventiveness, allows trial-error and the ability to retrace the path, and completely changes the attitude of students towards life processes. We have attempted this approach in M Sc Zoology at Pune but it is often too late because college students already have hardened the opposite mind-set and lost the work-culture due to easy marks, weak and repetitive courses, poor teachers, absence of an argumentative atmosphere, and absence of modernity. Therefore, attempts to resurrect a correct teaching sequence at M Sc can be only fractionally successful. In India, more than a quarter million students lose three precious and intellectually formative undergraduate years. Earlier, a matriculate could become a clerk, section officer or a school teacher which the present-day graduate cannot. The present educational system is not only chaotic but dumps the students in the rut of postponing entry in to professional life.

What can we do?

We have to implement drastic changes in the system and process of higher education. The correct format includes (i) a synthetic teaching sequence and multidirectional inputs in foundation courses, (ii) correct information, its verifiability and revision, (iii) continuous teacher training with a research base, (iv) efforts to develop manual, cognitive and combinatorial skills, (v) promotion of climate to learn to make mistakes and to correct these, (vi) cultivation of ability to learn to speak, read, write and listen, (vii) promoting contest, dissent expression and nurture of curiosity, and (viii) promoting ability to compare, verify and correct.

I propose a five-year integrated M Sc which would utilize the entire span to deliver life science graduates who can think, perceive, conceive, and copulate information and ideas into knowledge on a sustainable and constructive basis. The proposal eliminates the existing lacunae. Another such proposal suggested earlier³ lacked the conceptual framework, a systematic analysis of the reality, or tenable solutions. The 10+2 syllabi are far better than present the FY B Sc. In my proposal, students can take a B Sc after +3 and leave. A 5-year graduate will have comprehensive subject knowledge, a research base, creative inputs and innovative spirit with a hands-on experience in a research laboratory or field. They will communicate well in modern languages in life sciences, develop self-respect, confidence and fearlessness and maintain high level of curiosity. The system will also promote remedial education to students from rural and socially-deprived backgrounds. Only fully-trained graduates should be licensed to practice life sciences. With a 'micro to macro' synthetic attitude inculcated, it does not matter which specific subject and specialization the student pursues.

The five-year integrated M Sc programme in life sciences

After 10+2, all science subjects should teach similar +3 syllabi with emphasis on the development of communication skills, manual skills, interactive teaching, strong bias for field- and laboratory-training to teach the logic of science and concepts. The present analytical teaching cascade needs to be inverted as a natural mimetic to build a synthetic mind-set with readiness to contest through dissent, combativity for the satisfaction of the curiosity. The consequences of the synthetic approach would be dramatically different on the mind-set and temper. The exposure to management, accounting practices and patent laws will induce students to go into an operational mode for immediate employment in industry. They will become far better teachers and work-

force in science and technology, something that M Sc Biotechnology programme has achieved only marginally.

Summing up

I have evolved a streamlined structure (Table 1) for Life Science education with an exit point, but not entry, after the third year when the student is well trained in basic biology and skills required to promote their access to other professional outlets. The format differs significantly from the classical 3+2 pattern. Similar formats can be developed for other sciences by mini-packaging molecular biology and genetics, cell-developmental biology and immunology, biochemistry and bioenergetics, classical animal, microbial and plant studies, and environmental biology, but not by eliminating these⁴ so that students from other science streams can cross over to life sciences after +3. Students and teachers for such a course should have passed appropriate aptitude tests. Naturally, the present makers of the educational policy need dramatic reorientation, and perpetuation of the practice of 'mandarinage' has to be avoided. Here I refer to the proposal⁴ that ignores the reality of science teaching in India⁵. The process of education should not be under the control of those who are responsible for destroying our educational system as science education requires a level playing field. Otherwise, this model too will end up as the symbol of a failed trial which, at this point, is tantamount to accepting a pawn-status on the scene of rapid globalization. It will also fail if implemented primarily with part-time teachers, howsoever expert and good research scientists they may be, because a student needs continuity in the teacher-contact and a sense of belonging. The implementation will require an aggressive policy to recruit good teachers from within and outside India, to provide a congenial habitat, well-thought procedures for screening students and teachers, equal opportunity, a thoughtful marriage between private and public resources, updatable infrastructure, and methods to promote better performance in students from deprived and rural populace to match the constitutional guarantees without downgrading the educational standards. A good set-up for higher education cannot do away with the research component and must avoid inbreeding. If state governments do not have the will, then the higher education should be entrusted solely in the hands of the central government⁵. Finally, the confidence in the 80+ percentile group at 10+2 needs reassessment because most such students are also products of the so-called parallel education system which the poor students from the lower percentile groups cannot access. This is why a small but measurable proportion of late bloomers appears.

Table 1. Structure of a 5-year integrated M Sc course in life sciences

Year 1. Nuts, bolts and instability	
Semester I	
English for Scientists	Computing, MS Word, Excel, Access, Power point
Measurement theory	Principles of Instability – atoms and molecules
Chemistry–synthesis and catalysis	
<i>Catch-up courses</i>	
Semester II	
Basic electronics	Philosophy of Science – Logic
Statistics and applied mathematics	Biochemistry
<i>Catch-up courses</i>	
Year 2. The cascade of complexity: transmission and the drive	
Semester III	
Molecular Biology	Materials and polymers
Cell Structure and Physiology	Bioenergetics
<i>Catch-up courses</i>	
Semester IV	
Microbial structure, function and taxonomy	Principles of genetics
Developmental biology	Immunology
Carpentry/machine shop/drafting/photography	
<i>Catch-up courses/Year-end summer project</i>	
Year 3. The body	
Semester V	
Structure and function relationships in plants	Structure and function relationships in animals
Animal and plant taxonomy and informatics	Science instrumentation
Biochemical techniques	
<i>Catch-up courses</i>	
Semester VI	
Evolution	Data bases and information retrieval
Ecology	Biophysical techniques
Microtechnique, morphometry and image processing	
<i>Catch-up courses/Year-end summer project</i>	
Year 4. The road and destination	
Semester VII	
Any one among specialized streams, theory and lab rotation	Chronobiology
Optional courses (any two)	Research project and seminars
<i>Catch-up courses/Year-end summer project</i>	
Semester VIII	
Specialized stream theory and lab rotation	Genetic engineering and gene transfer
Optional courses (any two)	Research project and seminars
<i>Catch-up courses/Year-end summer project</i>	
Year 5. Occupational skills	
Semester IX	
Specialized stream theory and lab rotation	Optional courses (any two)
Management	Research project and seminars
<i>Catch-up courses</i>	
Semester X	
Specialized stream (lab rotation)	Optional courses (any two)
Structure of human society, interactions, eugenics	Research project and seminars
<i>Catch-up courses/Year-end summer project</i>	

Contd...

GENERAL ARTICLE

contd...

Specialized streams

Animal tissue culture, vaccines and diagnostics
Molecular biophysics
Plant genetics and breeding
Cell and developmental biology
Computer interactive and audio-visual methods in research and education
Ecology and environmental management
Mycology and plant pathology
Comparative plant anatomy, systematics, community structure and management of the flora
Industrial microbiology.

Optional courses

[excepting those included in a special stream]

Genetic toxicology
Developmental genetics
Physical anthropology

Physiology of reproduction
Neurobiology
Electron microscopy

Pest control
Animal-plant interactions
Applied entomology

Mushrooms and mushroom culture
Medicinal plants
Biopesticides and biofertilizers

Wild life management and conservation
Palaeobotany
Animal behaviour

Biochemical processes
Cell engineering
Fermentation technology
Museum management
Finance and marketing
Communication in Indian languages
Virtual reality.

Physiology and metabolism
Plant tissue culture, plant propagation
Molecular biology and genetics
Nutrition, eugenics and anthropology
Microbial systematics, community structure
pathology and management
Membrane biology
Entomology
Animal comparative anatomy, systematics, community structure, behaviour and management of the fauna
Neurobiology

Phage and viral genetics
Genetic engineering
Behavioural genetics

Endocrinology
Clinical microbiology
Toxicology

Epizootology
Insect biochemistry and physiology
Bioelectronics

Horticulture
Phytochemistry and plant products
Plant propagation

Land management
Palaeontology
Remote sensing

Enzyme engineering
Biomes culture
Cryobiology
Copyright and patent law
Computer graphics and modelling science
Foreign language proficiency: Japanese or German or French or Spanish or Russian or Arabic

Teaching methodologies. OHP, 35 mm slides, video, CD-ROMs, interactive computer-based teaching (CBT), laboratory and field work, electrical-electronics-mechanical workshop, seminars, tutorials, visits, summer and autumn training programmes, remedial courses.

Examinations. Objective, short and long answer, seminar, open-book, CBT

Funding. Central and state government, private

Infrastructure. Water, electricity, space, equipment and waste disposal

Faculty and students. Aptitude-tested.

Post-script

Unfortunately, our funding agencies lack the correct attitude for critically assessing mission-oriented projects, guts to make mid-course corrections and 'candour' to terminate open-ended and failed projects, be these in universities or national institutes. Most funding reaches a handful few without emphasizing either cost-benefit ratio or the productivity, and that it is almost never terminated despite failures. The applicants often present unattainable objectives using a rhetoric heard sympa-

thetically by the so-called peers who play the musical chairs in a dual role as peers and applicants, and subvert the entire process by breeding rampant nepotism. We really do not have a critical mass of peers for a meaningful peer-review in most hi-tech areas, or low-tech but multifaceted disciplines. Most members of the so-called expert panels do not even read most project proposals. Who, in India will assess proposals, say, from IISc? Who will stop the February gold-rush to Delhi? How can one eliminate the parochialism in research committees? In the West, peer review committees change

through rotation at intervals. We should recruit expert-help from outside, an idea vehemently opposed by the 'mandarins' of Indian science. At present, there is no independent audit of individual or mission-oriented research projects. Like in finance and accounts, the 'spender' cannot be the 'auditor'.

UGC and all Government agencies and NGOs involved in science and technology development in India should support this 'experiment' because it is revolutionary and original. It is a direct attempt to replant the cured soil of higher education in science with an epigenetically modified and better-yielding variety. Only after success in such efforts would we deserve the status of the third largest work-force in science and technology. Indeed, it is worth rerouting this work-force and even decreasing it because the quality, rather than quantity, is relevant to the process of nation building at this point. Even if the part dealing with the +3 level is implemented, the scenario for life sciences will change dramatically in India. A 5-year M Sc programme is not

another band-wagon to attract 'good' students. It must transcend geo-political considerations. Indian teachers are parochial and hate mobility. Then, let students move, as they do, to the training site. The psychological 'fix' by a 5-year M Sc⁶ will automatically curtail the use of students as the 'manual labour' for the peers practising science by 'proxy'.

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2. Sitaramam, V., *Curr. Sci.*, 1991, **60**, 537-540.
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6. Estimated cost: Rs. 4.5 crores for capital expenditure, Rs. 50 lakhs/year recurring, an intake of 40-50 students/year, and an endowment of Rs. 4 crores to cover salaries (1995-96 scales). Methods for aptitude tests are being studied.

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REVIEW ARTICLE

Progress towards malaria vaccine

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Resurgence of malaria has reached alarming proportions. The situation has become worse because of the widespread resistance to anti-malarials. Thus malaria vaccine research has become an area of intense activity even though host-parasite interactions are not well understood. Several antigens from different stages of the life cycle of malaria parasite have been identified. It is now clear that both antibodies and cellular immune responses are involved in malaria immunity. At the

same time it is becoming clear that plasmodium has developed exquisite mechanism to evade the immune responses mounted by the host. All kinds of vaccine constructs, based on recombinant antigens, synthetic peptides, and direct use of DNA are being attempted and several of these are undergoing human trials. Results of these trials and other research works clearly indicate that malaria vaccine development is hugely complex and success may not come easily.

MALARIA continues to be a major cause of morbidity and mortality in the tropical and subtropical areas of the world where approximately 350 million malaria cases occur each year. More than two million children die of the disease annually. The overwhelming success of chloroquine as a drug and DDT as an insecticidal, introduced in 1960s resulted in an impression that malaria could be controlled to a large extent if not eradicated. Unfortunately, resistance of the human malaria parasite *Plasmodium falciparum* to anti-malarial drugs and to its mosquito vector to insecticides has led to an alarming situation and drugs-resistant strains of *P. falciparum* have spread throughout the tropics. Given the fact that

no new anti-malarial drugs or new insecticides superior to DDT are likely to be available in the near future, the disease situation is becoming hopeless.

Vaccine should be a useful addition to chemotherapy and the vector control program in malaria control. However, until recently there was no way to obtain sufficiently large amounts of antigenic material from the parasite. With the availability of *P. falciparum* in culture form, it has become possible to study the molecular basis of the parasite function. Identification and production of antigens involved in protective immunity by applying tools of modern biology has changed the direction of malaria research, particularly the vaccine development.

There are four species of human malaria: *P. falciparum*, responsible for practically all malaria deaths, *P. vivax*, also widespread and the cause of considerable morbidity, and the less prevalent species *P. ovale* and *P. malariae*. The parasite undergoes a complex life cycle. There are three major stages in the life cycle and each of the developmental stages is morphologically and antigenically distinct and there appear to be several antigenic molecules at each stage that could be potential vaccine targets.

Malaria vaccines against the three distinct developmental stages of the parasite are being developed^{1,2}. Vaccines against the pre-erythrocytic stages of malaria aim to eliminate infection by blocking sporozoites from entering hepatocytes or by destroying the infected hepatocytes. The second type of vaccine targeted against the blood stages of the parasite which would be expected to prevent the disease or significantly reduce the parasite load, and therefore the intensity of infection. The third type is aimed at the sexual stages of parasite and aims to limit transmission of the disease. An altogether different kind of vaccine, namely anti-disease vaccine, has also been proposed³. Such a vaccine would aim to neutralize factors responsible for the pathology associated with malaria infection. Keeping in view our own research in the malaria research group at ICGEB, the main focus of this review will be on sporozoite and blood stage based vaccines.

Despite tremendous progress in malaria research during the past twenty years which has led to a vast amount of information, the exact nature of malaria immunity and effector mechanisms involved remain ill understood. Malaria immunity is slow to acquire and is usually short-lived. Thus, children up to 5 years of age are most susceptible to severe clinical disease; adults show little parasitaemia and rare clinical disease. The highly polymorphic nature of malaria antigens within each parasite species and antigen structure are proposed to be the main reasons for the slow and transient nature of the acquired immunity. Further, the immunity is both species- and stage-specific. A person with immunity to *P. falciparum* may still be susceptible to *P. vivax* infection and vice versa. Similarly, immunity to the sporozoite stages may still leave an individual susceptible to the blood stage infection. It is often thought that a successful malaria vaccine will have components from several antigens belonging to the different stages of the parasite. However, non-availability of a suitable animal model for human malaria will remain a major hurdle in the development of malaria vaccines.

Is there a rationale for a malaria vaccine?

Given the complexity of the parasite's life cycle, complicated and ill-understood host-parasite interactions

and several mechanisms that are operative in favour of the parasite to evade immune responses, it does appear that development of an antiparasite vaccine will be a very difficult task, if not an impossible one. On the other hand, there are several reasons to believe that malaria vaccine will be developed in future. Some of these are:

1. Individuals living in malaria endemic areas do develop specific immunity that decreases the parasite's ability to survive in the human host and also decreases the clinical consequences of infection. However, in contrast to rapid and long-lasting immunity induced during viral infections, malaria immunity takes a long time to develop and is usually short lived in absence of continued exposure to infection. Clearly a successful malaria vaccine will have to be more effective at inducing immunity than the natural infection.

2. Transfer of immunoglobulin from individuals with acquired malaria immunity, to naive humans almost completely protected them from infection⁴.

3. Immunization with irradiated sporozoites induces a solid immune protection in animals^{5,6} and in humans⁷. However, only intravenous deposition of irradiated sporozoites, not a convenient route for general vaccination, provides optimum protection.

4. Significant levels of protection were observed in early trials when purified malarial antigens were used as immunogens^{8,9}. More recently, several trials with recombinant antigens have shown high levels of protection against challenge with blood stage parasites^{10,11}. This is encouraging for vaccine developments since only a few antigens, out of the numerous suggested have actually been tested in primates. It is very much possible that valuable vaccine antigens will be discovered and used as vaccine candidates.

Nature of malaria vaccines

Most successful vaccines have been based on attenuated or killed pathogens. Since human blood is needed for *P. falciparum* culture and *P. vivax* has not yielded to culture at all, the focus in malaria vaccine development is largely limited to well-defined molecules which can induce protective immune responses and now be easily produced by recombinant DNA techniques in various systems. While a recombinant hepatitis B vaccine is already in use in humans, several other recombinant vaccines are in the developmental stage.

The fact that immune response against short peptide can produce neutralizing antibodies against viral and other proteins, has opened way to the development of synthetic peptides as immunogens for vaccination¹². But short peptides are usually poor immunogens, and the carrier proteins that are traditionally used to enhance their immunogenicity have inherent problems associated

with their use¹³. Use of T cell determinants in place of carrier proteins is being investigated¹⁴⁻¹⁶.

Viral vectors including vaccinia and salmonella have been considered as vehicles for carrying the gene of a target antigen. More recently, direct DNA immunization has also been attempted in malaria vaccine development¹⁷. The main attraction of developing viral vectors or direct DNA immunization protocols is that it obviates the use of adjuvants which are necessarily needed in case of recombinant proteins and synthetic peptides.

Liver stage antigens

The early observation that immunization with irradiated sporozoites could protect rodents or humans against challenge with viable sporozoites provided the basis for much of the work on the sporozoite stage vaccine⁵⁻⁷. It is now clear that both humoral and cell-mediated immune functions contribute to the acquired immunity in man. In rodent model system also, antibodies to the circumsporozoite protein, CD⁴⁺ cells and CD⁸⁺ T cells have all been implicated in protection. Of the liver stage proteins, the circumsporozoite (CS) protein has primarily been the focus of most studies in both rodents and humans¹⁸.

The CS protein covers the whole surface membrane in mature salivary glands, of all malaria species sporozoites and is believed to be involved in the process of sporozoite's penetration into hepatocytes. All CS proteins have an immunodominant epitope in the middle region formed by tandem repeats of amino acids, which vary in sequence among different species of malaria parasites. It was observed that antibodies to the epitopes formed by the repeats mediate protection against sporozoite-induced malaria. This led to the first generation of subunit malaria vaccines to be tried in humans. Two candidate vaccines based on (Asn-Ala-Asn-Pro) repeats were developed. One of these was a recombinant polypeptide (R32tet32), containing thirty two *P. falciparum* CS repeat units, fused to a sequence of 32 amino acid residues from the plasmid vector. The other vaccine consisted of synthetic dodecapeptide, (Asn-Ala-Asn-Pro)₃, which represents the epitope recognized by antibodies in the sera of humans living in endemic areas, conjugated to tetanus toxoid. However, both the constructs were poorly immunogenic in humans, when administered in alum^{11,19}. Limited protection which correlated with the levels of anti-repeat antibodies was observed but by and large these trials were considered unsuccessful. Similarly in another human trial, poor immunogenicity was again observed when a recombinant CS protein of *P. vivax* was administered in alum²⁰. It was soon realized that a successful anti-sporozoite vaccine will have to induce high antibody levels to the repeat sequence with the appropriate specificity. It is

now clear some parasite antigens like the CS protein are poorly immunogenic because the helper T-cell epitopes lie mostly in the polymorphic region of the protein. A vaccine dependent on a foreign carrier protein to provide T-cell help may provide high primary antibody response, but will not respond to subsequent parasite challenge, and therefore will not be effective unless administered repeatedly.

The target for cellular immunity can be any antigen, surface or internal of the parasite, containing one or more T-cell epitopes. Sensitized T-cells may secrete γ -interferon or other lymphokines including tumour necrosis factor or oxygen species which are known to kill parasites²¹. Further, cytotoxic T-cells (CD⁸⁺), in addition to activating the non-specific effector functions, may also recognize specific epitopes on parasite-infected hepatocytes and kill them. While T cell responses to CS protein have been studied in some detail, recently, two other liver stage antigens namely, the sporozoite surface antigen 2 (SSP-2) and the liver stage antigen 1 (LSA-1) have been characterized and their immune responses in humans and in animals are being evaluated, in order to develop these antigens as possible vaccine candidates^{22,23}.

Asexual blood stage antigens

Antibody response to the asexual blood stages of malaria parasites is extremely diverse. A very large number of polypeptides are recognized by antibodies in the serum of malaria-infected individuals. Selection of suitable candidate proteins from a very large number of antigens is itself a major difficulty in the development of a blood stage malaria vaccine (Table 1). Since the titre of antibodies to the blood stage antigens does not correlate with protection, it is quite possible that most antigens do not produce protective responses. The immediate goal, therefore, is to identify the antigens capable of inducing protective immune responses.

Non-availability of a suitable animal model is another major block in the way of malaria vaccine development. Although a vaccine target antigen for *P. falciparum* can be tested in Aotus or Saimiri monkeys, the infection in monkeys takes somewhat different course than the human disease. Moreover these monkeys are not easily available. For these reasons, indirect criteria such as location and the possible function of the antigens are often used in choosing a possible protective antigen. Some of the major vaccine target antigens from the blood stages of the malaria parasites are briefly described below.

Most protection studies with recombinant polypeptides fragments or chemically synthesized hybrid peptides have focused on a relatively small number of antigens. Major surface protein 1 (MSP-1) was one of

the first antigens identified as a potential vaccine candidate. It contains variable and highly conserved regions and is synthesized as a large protein, anchored to the merozoite surface and undergoes processing in two steps. Recombinant fragments representing the MSP-1 conserved regions were found to induce partial protection in Saimiri monkeys²⁴. That immunogenicity of peptide fragment can be highly enhanced by covalently attaching it to suitable T helper cell epitopes was shown in case of a conserved MSP-1 fragment, and higher levels of protection were observed in monkeys with such a construct²⁵. A significant result of these studies was the speculation that the protection was at least partially T-cell mediated. We have also characterized B and T-cell epitopes in the conserved regions of MSP-1 and have used these epitopes in the design of multiple epitope peptides²⁶. The C-terminal 19 kDa fragment, highly rich in cysteine residues, which are found conserved in several species of *Plasmodium*, appears to be crucially involved in merozoite invasion of erythrocytes and is carried into cells by invading merozoites²⁷. Phase one and phase two clinical trials using this fragment are being planned in the USA.

The second merozoite surface protein (MSP-2) is a 45 kDa protein located on the surface of the merozoite. The molecule contains a variable central repeat region, but the N and C terminal regions are highly conserved. Synthetic peptides from these conserved regions of Pf MSP-2, conjugated to a carrier protein, provided significant protection against *P. chabaudi* challenge in mice²⁸. Phase I trials with a combination of MSA-2 and the CSP protein have been conducted in Australia. Apical membrane antigen (AMA-1) is another protein that is highly conserved in all species of plasmodium analysed so far²⁹. Monoclonal antibodies against this antigen inhibit merozoite invasion of red cells and currently this antigen is undergoing human trials as a vaccine candidate. Genes for blood stage proteins like the acid base rich antigen (ABRA), the ring-infected erythrocyte surface antigen (RESA), the serine rich antigen (SERA), erythrocyte binding antigen (EBA-175) and several other malaria antigens (Table 1) have been cloned and sequenced³⁰. These antigens are at different stages of development for their potential as vaccine target antigens^{30,31}. Immunization studies with these antigens, produced by recombinant methods have resulted in a wealth of information which ironically reveal that malaria immunity might be more complex than thought earlier. As a result of this it is now believed that an approach of combining several conserved antigens in a cocktail vaccine might be more successful in addition to providing a means to reduce the risk of the selection of vaccine resistant forms of the parasite. An attenuated live vaccinia virus-based vaccine consisting of seven candidate antigens from the different life stages of the parasite, known as NYVAC-7, is being tested in humans for safety and immunogenicity in the USA.

Synthetic peptides as malaria vaccines

An alternative to the use of full length recombinant or native antigens is to identify the peptide epitopes on immunogens which induce a protective response and to use synthetic versions of the peptides in the production of vaccines. This approach seems particularly suited for parasitic diseases where it is increasingly being realized that some crucial epitopes of vaccine target antigens remain cryptic during immunization with the native antigens.

A synthetic peptide-based polymer, termed as SPf 66, developed by M. E. Patarroyo in Colombia, has undergone extensive human trials in several locations in Latin America and more recently in Africa and South East Asia³²⁻³⁵. The basic unit of SPf 66 as a hybrid peptide represents epitopes from CS protein, MSP-1 and two other sequences from yet-to-be-characterized 35 kDa and 55 kDa proteins from the blood stages of the parasite^{36,37}. Following the protection experiments in monkeys, the first human trials with SPf 66 were conducted in South America where high levels of protection were reported³³. These and subsequent results from a field study conducted in Tanzania confirmed that the vaccine was safe, induced anti-SPf66 antibodies and was able to provide up to 30% protection in the immunized children³⁴. However, more recent and carefully-designed studies conducted in The Gambia and in Thailand have shown³⁵ that SPf 66 could not provide any protection at all. It is now more or less accepted that this vaccine is not protective in areas of high malaria endemicity and that further efficacy trials are not warranted³⁵. This has been a major setback to malaria vaccine development and the question as to why SPf66 has failed after initial promise of partial protection does become a relevant one, in particular with respect to field trials involving future vaccine candidates.

The development of SPf66 has been full of controversies, ranging from scientific criticism to the issue of attitudes towards research carried out in a developing country. M. E. Patarroyo published in 1988 his findings that SPf66 was partially protective against *P. falciparum* infection in both Aotus monkeys and humans^{36,37} and since then insisted that SPf66 is a viable malaria vaccine. His enthusiasm has never been shared with vigour by other scientists³⁸ which was partly due to the fact that two independent trials with SPf66 in monkeys failed completely^{39,40}. The protection observed in large field trials in Latin America was also subjected to debates on the basis of inadequate epidemiological design of these trials and claims that SPf66 could also protect against *P. vivax* infection; the latter certainly appeared implausible. These arguments were certainly rational and intellectually justifiable. However, it should be pointed out that the same arguments could equally well apply to several clinical and field trials, including some peptide

based malaria vaccines, performed by scientific institutions in the developing world who were critical of Pataroyo's claims. Looking back, however, it does seem that the simple approach of attempting to produce a minimalist mimic of the malaria parasite was ambitious, just as it must be said once again, were the human trials conducted in the USA with the synthetic constructs based on repeat structures of the CS protein. Doubts about the design of the earlier field trials with SPf66 were, however, removed in the later trials done in collaboration with WHO/TDR in Tanzania. But the most puzzling observation in all these human trial studies^{33,34} was the fact that while SPf66 produced antibodies upon immunization, it had no correlation with the observed protection^{33,34} was being achieved. Perhaps some unknown protective mechanisms were involved. In the end, however, the inability of other workers to reproduce some of the key results described for SPf66, along with the results of the recent human trials in The Gambia and Thailand does seem to suggest that there is little chance that it will be used as a vaccine against malaria. Be that as it may, the development of SPf66 has certainly established that a chemically synthesized peptide construct can be a safe immunogen in humans, and has opened way for the development of other peptide-based vaccines.

Malaria research at ICGEB

Some of the major malaria antigens have within their structures regions that are highly conserved, not only within the different strains of the parasite but also among different species of malaria parasite. It has been argued that it is most meaningful to base vaccine constructs on the regions that have remained conserved under immunological pressure; such sequences are more likely to represent functional domains of the parasite surface proteins. My research group at ICGEB has followed this approach to develop synthetic peptide immunogens as malaria vaccine candidates, to be initially tested in animals.

A special feature of most malaria proteins is the presence of immunodominant repeat peptide structures⁴¹. There is also extensive antigenic cross-reactivity reported in malaria and these factors appear to be involved in immune evasion mechanisms developed by the parasite⁴². To probe if the repeat structures contribute to the observed cross-reactivity among the known antigen of *P. falciparum*, we synthesized peptides based on repeat structures from different antigens of *P. falciparum* and were able to show in a specific manner that the humoral immune responses to these peptides are highly cross-reactive⁴³. Circular dichroism studies on these peptides revealed that these peptides tend to adopt helical structure in solution. However, it is not clear if the

preference of repeat peptide structures for a given secondary structure alone is responsible for the observed immunological cross-reactivity³⁶. Interestingly, we found that the peptides based on *P. vivax* repeat structures did not show any significant cross-reactivity with the *P. falciparum*-based peptides (unpublished work, Chauhan *et al.*). It is now firmly believed that highly immunodominant repeat structures are utilized by the parasite to evade immune responses mounted by the host. It is also being suggested that epitopes crucial for the parasite survival may remain mostly hidden, and although functionally relevant, they may not be as accessible to the immune system of the host as the structurally dominant repeat structures. Delineation of such conserved, perhaps cryptic, epitopes may be necessary to the design of a peptide-based malaria vaccine.

The epitopes recognized on a protein may be linear or spatial: B-cell epitopes, involved in the recognition of antigens by antibody, may be either linear or discontinuous, but the T-cell epitopes, involved in cell-mediated immunity and T helper activities, are invariably linear. Peptides themselves are poor immunogens and usually need to be conjugated to a suitable protein to obtain an immune response. We were able to show that immunogenicity of a B epitope from Pf MSP-1 could be enhanced by linking it covalently with a T-cell epitope sequence from the CS protein or tetanus toxoid¹⁶. There is increasing evidence that highly enhanced and specific immune responses can be obtained by combining B-cell epitopes with Th epitopes although

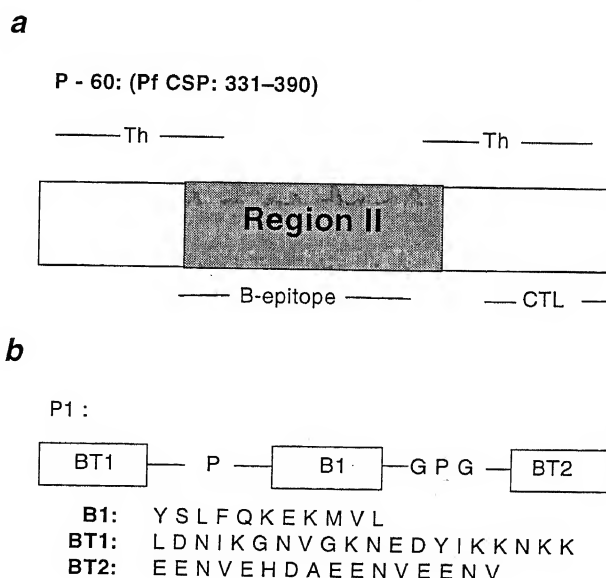


Figure 1 a, b. Schematic representation of P60 and P1 indicating the location of different epitopes. **a**, At the N-terminus of P60 is a Th epitope (amino acid residue no. 331-349 of the CSP) overlapping a B epitope in region II (residues 346-363 of the CSP), followed by another Th epitope (362-381) and an overlapping CTL epitope (368-390). **b**, BT1 and B1 are peptide sequences from MSP-1 whereas BT2 is a peptide sequence from RESA in a hybrid peptide P1.

Table 1. Asexual stages vaccine target antigens

Antigens	Approx. size	Location
Sporozoite/liver stages		
Circumsporozoite surface protein (CSP)	60 kDa	Sporozoite surface
Sporozoite surface protein-2 (SSP-2)	63 kDa	Sporozoite surface, Micronemes
Liver stage antigen-1 (LSA-1)	200 kDa	Parasitophorous vacuole
Sporozoite threonine asparagine rich protein (STARP)	70 kDa	Sporozoite surface
Blood stages		
Merozoite surface protein-1 (MSA-1)	195 kDa	Merozoite surface
Merozoite surface antigen-2 (MSA-2)	45 kDa	Merozoite surface
Apical membrane antigen-1 (AMA-1)	83 kDa	Rhoptry organelle
Rhoptry antigen protein-1 (RAP-1)	80 kDa	Rhoptry organelle
Rhoptry antigen protein-2 (RAP-2)	42 kDa	Rhoptry organelle
Ring erythrocyte surface antigen (RESA)	155 kDa	Dense granules
Acid base rich antigen (ABRA)	75 kDa	Parasitophorous vacuole
Histidine rich protein-2 (HRP-2)	65 kDa	Secreted into plasma
Serine repeat antigen (SERA)	110 kDa	Released at rupture
Pf Erythrocyte membrane protein-1 (PfEMP-1)	250–400 kDa	Parasitized erythrocyte surface
Erythrocyte binding antigen-175 (EBA-175)	175 kDa	Micronemes/Apical end
Thrombospondin related anonymous protein (TRAP)/(SSP-2) ?	63 kDa (?)	(?)

Table 2. Multiple epitope peptides developed at ICGB

Peptide	Sequence	Antigen
P1	LDNIGNVVGKNEDYIKKNKKPYSLFQKEKMVLGP GEENV EHD A EENV EENV	MSP-1/RESA
P18	EWSPCSVTCTGNGIQVRIK	CSP
P32	IEQYLKKIKNSISTEWSPCSVTCGNGIQVRIK	CSP
P60	IEQYLKKIKNSISTEWSPCSVTCGNGIQVRIKP GSANKPKDEL DYENDIEKKICKMEKCS	CSP

the manner in which this can be done still needs to be worked out. Design of synthetic peptides containing disease relevant B and T epitope sequence in order to generate specific immune responses is central to the development of peptide vaccines.

Carboxy-terminal to the repeats, CS proteins from all *Plasmodium* species possess a conserved region (region II) centered around a cysteine containing nonapeptide sequence, WSPCSVTCTG, also found in proteins involved in cell-cell interactions, such as thrombospondin, properdin, etc. Results from our laboratory and the works of others have clearly indicated that region II is a sporozoite ligand for the hepatocyte receptor^{44,45}. We were able to show that two peptides, based on the *P. falciparum* region II sequence, P18 and P32 (Table 2) significantly inhibited *P. berghei* sporozoite invasion into HEP-G2 cells⁴⁵. Quite significantly, we also found that antibodies to P32 also inhibited *P. berghei* sporozoite invasion of Hep-G2 cells. Since P32 contains a strong T-cell determinant in addition to the conserved region II motif, we immunized mice with P32 without using a carrier protein. Significant immune re-

sponses were obtained in two different strains of mice. The fact that immunization of mice with P32, without the use of a carrier protein, protected them against a lethal challenge of *P. berghei* sporozoites strongly suggests that P32 contains crucial B and T-cell epitopes and that region II may be useful as a component of a malaria vaccine⁴⁵.

Surprisingly the conserved nonapeptide sequence of the region II is also present in another sporozoite surface antigen called thrombospondin related adhesive protein (TRAP or SSP-2), which also has been recognized to play a crucial role in the sporozoite invasion of hepatocytes⁴⁶. Although first described from the blood stages, the expression of TRAP during the erythrocytic stages of the parasites has been controversial⁴⁶. We have found that antibodies raised against a synthetic peptide containing the conserved nonapeptide motif recognized a protein in the blood lysate of *P. falciparum* culture⁴⁷. Immunoprecipitation experiments with antibodies raised against recombinant TRAP, and its fragments, provided further evidence for the presence of a TRAP-like protein during the blood stages of *P. falciparum*. Further, we

found that anti-peptide antibodies inhibited merozoite invasion of erythrocytes⁴⁷. Mice immunized with synthetic peptides P32 or P60 (Table 2), both of which contain several B and T epitopes (Figure 1a) including the region II sequence, were partially protected against a heterologous challenge with blood stage parasites of *P. yoelii*⁴⁸. Immunization with P60 in rhesus monkeys also produced high anti-peptide antibody response. Protection experiments in Aotus monkeys with P60 have been planned. Fine specificity of the immune responses to these peptides with respect to various B and T-epitopes showed that the response was focused on the region II sequence. From the cytokine analysis data we observed that both Th1 and Th2 cellular responses were induced upon immunization with the peptides⁴⁸. Our results clearly show that through the use of appropriate peptide it may indeed be possible to focus the immune response to the regions which otherwise remain largely cryptic during the course of natural infection or upon immunization with recombinant or native proteins.

It is generally believed that a future malarial vaccine will need to contain a combination of different antigens. Synthetic peptide constructs containing B and T-epitopes from different antigens have been developed, but a major concern with epitope-based constructs is the genetic restriction of the immune response in an outbred human population. Characterization of B and T-cell determinants in a viral or parasite antigen may be relatively simple now, given the advances in peptide synthesis, theoretical prediction of antigenic sites and a battery of simple assays. We have analysed immune responses to a series of synthetic peptides representing predicted B and T-cell determinants from conserved regions of MSP-1, TRAP, RESA and AMA-1. Analysis of the development of immune response to specific immunodominant peptide fragments from different major malaria antigens revealed a direct correlation of malaria-specific antibodies with transmission of the disease²⁶. One of the peptides, representing repeat structure peptide of RESA has turned out to be a good marker and may be used as a capture antigen in ELISA to determine the status of malaria control programme⁴⁹.

Based on B and T-cell epitopes of liver and blood stage antigens, CS protein, and MSP-1 and RESA, respectively, two multiple epitope peptides, P1 and P2 (ref. 50) were designed and synthesized (Table 2). These linear peptides were highly immunogenic in mice without the use of a carrier protein and both peptides were able to induce cellular proliferative responses⁵¹. We also studied the effect of different adjuvants on the immunogenicity of these peptides and found that in alum also these peptides are excellent antigens. Interestingly immunization with P1 (Figure 1b) provided protection to BALB/c mice against a heterologous, *P. yoelii* challenge infection. However, P2, which also contains similar B and T epitopes, but in different orientation, failed

to provide any protection. We found that in case of P2 immunization the humoral response was focused on T-epitope sequence instead of the B-cell determinant⁴⁴. Our results suggest, as indeed from several other groups working on peptide vaccines, that while linear multiple epitope peptides may offer attractive alternatives as subunit vaccines, it is clear that ground rules for the design of such immunogenic peptides are yet to be defined⁵¹⁻⁵³.

It is obviously important to analyse whether the epitopic sequences from malaria antigens are immunogenic in the context of infected individuals living in malaria-endemic areas. Even though most of our work involves the use of highly-conserved regions of the vaccine target antigens, it will be of relevance to characterize these antigens from Indian isolates of *P. falciparum*; surprisingly none of these antigens have been characterized as yet. As a step in this direction we have isolated, cloned and sequenced the gene of the MSP-2 from three geographical isolates from different malaria-endemic areas in India. The MSP-2 gene of Indian isolates is remarkably similar to the FC-27 strain from Papua, New Guinea⁵⁴. We are now in the process of characterizing genes of MSP-1, CS protein, TRAP and AMA-1 of *P. falciparum* from Indian isolates.

P. vivax/P. cynomolgi malaria

Of all the malaria cases in India up to 60 to 70% are due to *Plasmodium vivax* infection and although *P. vivax* infection does not directly kill the host, it causes a great deal of discomfort and morbidity. Relatively less is known about the immune responses of the host to *P. vivax* infection. Also, the antigens of *P. vivax* are not as well characterized as *P. falciparum*, mainly because unlike *P. falciparum*, *P. vivax* has resisted all attempts of culturing the parasite; infected humans remain the only source of the parasite material. *Plasmodium cynomolgi* is a simian malaria which is closely related to *P. vivax* in taxonomy and morphology, and is regarded as a good model to study the *P. vivax* infection⁵⁵. We have characterized genes of three major vaccine target antigens from a *P. cynomolgi* isolate, obtained from the Central Drug Research Institute, Lucknow, in order to test their potential as vaccine candidates in the readily available rhesus monkey model. We found that the AMA-1 of *P. cynomolgi* is closely related to the *P. vivax* counterpart, and most structural features like number and position of cysteine residues and proline residues are conserved in the two homologues⁵⁶. The full length AMA-1 and a truncated version of it have been expressed in *E. coli* and baculovirus system, in our laboratory. This will allow us to investigate the immune responses to Pc AMA-1 in the monkey model, and thus to analyse its role in protective immunity during the blood stages of the parasite.

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Another important vaccine target antigen, TRAP, is also a major focus in our laboratory for analysing its role in malaria immunity. We have observed that certain highly conserved B-cell epitopes of the Pf TRAP remain cryptic during the course of natural infection. The ability to make important functional epitopes cryptic appears to be a general strategy of parasites to evade host's immune responses as has been shown in case of surface protein of *Trypanosoma cruzi*⁵⁷. We have cloned and expressed in bacteria the full length TRAP gene of *P. falciparum* from an Indian isolate, and its fragments, representing the N and C-terminal domains of the protein. Since TRAP is considered one of the most conserved malaria antigens, we have also characterized the TRAP gene from *P. cynomologi*⁵⁸. The potential of this antigen and their fragments in protection will be tested in a relevant animal model. Since TRAP is highly conserved across the *Plasmodium* species, the results of *P. cynomologi* experiments will be of direct relevance in evaluating its role in *P. vivax* and *P. falciparum* vaccine development.

As mentioned earlier, it appears that the ability to block the development of immune responses to the functional epitopes is one of the main strategies to evade immune responses of the host. This is why a synthetic peptide-based vaccine development approach may be most relevant in a parasite disease like malaria. Through the use of peptides it may be possible to focus immune responses to the otherwise cryptic epitopes; development of synthetic peptides as immunogens is, therefore, an immediate need for parasite vaccines. However, genetic restriction of immune response, specificity of epitopic sequences, use of appropriate adjuvants, etc. are some of the problems that will have to be sorted out before peptides may be useful for vaccination. With the availability of recombinant antigens and their fragments in our laboratory, it should now be possible for us to compare the immune responses obtained upon immunization with whole antigens with those obtained from the use of synthetic, multiple epitope peptides. For the development of vaccines against parasitic diseases, this appears to be one of the most important questions.

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RESEARCH ARTICLES

What's the essence of royalty – one keto group?

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A honey bee colony consists of a single queen, tens of thousands of nearly sterile female workers and usually a few hundred drones. The presence of the queen inhibits rearing of new queens, stimulates foraging and interaction of workers with the queen and, (along with the queen's brood), inhibits the development of worker ovaries. Most or all of these effects of the queen on the workers are mediated through primer pheromones secreted by the queen. An important component of the queen's pheromone blend is 9-keto-(E) 2-decenoic acid (9-ODA). Workers also produce related substances which appear to function as nutrients and food preservatives. A dominant component of the worker blend is a diacid which is made from a precursor molecule hydroxylated at the ω

carbon atom rather than at the $\omega-1$ carbon atom. The $\omega-1$ precursor is used by the queen which leads to the formation of a keto acid. One might say that the fundamental difference between a queen and worker, the essence of royalty is therefore, one keto group! The recently-elucidated caste-specific biosynthetic pathway for the production of these pheromones permits two other speculations. One is that workers can be thought of as being closer to the ancestral solitary condition and that queens can be thought of as a derived invention of sociality. The other is that, compared to non-social species, social insects are especially predisposed to evolve novel structures and characters as exemplified by the queen for example, through the process of evolution of gene duplication.

Honey bees

WILLIAM Morton Wheeler¹ says of the honey bee: 'Its sustained flight, its powerful sting, its intimacy with flowers and avoidance of all unwholesome things, the

attachment of the workers to the queen – regarded throughout antiquity as a king – its singular swarming habits and its astonishing industry in collecting and storing honey and skill in making wax, two unique substances of great value to man, but of mysterious origin,

made it a divine being, a prime favourite of the Gods, that had somehow survived from the golden age or had voluntarily escaped from the garden of Eden with poor, fallen man for the purpose of sweetening his bitter lot.' Today one might add, 'and also for the purpose of providing him with a model system to study everything he wishes to know about animals'. Honey bees have served as an excellent model system in animal behaviour, sociobiology, physiology, genetics and biochemistry. Above all the discovery by Karl von Frisch², of colour vision in the honey bee and of its ability to use ultraviolet as another colour and see patterns in flowers that we cannot, and his elucidation of the dance language of the honey bee have created in us a new respect for animals. In the words of J. L. Gould, 'The lesson is a melancholy one. We are blind to our own blindness and must not try to read our disabilities into the rest of the animal kingdom.' Now honey bee queens and workers are on the threshold of teaching us the essence of royalty and the lesson is an even more melancholy one – the answer seems to be, one keto group!

The queen pheromone

Honey bees live in populous colonies consisting of tens of thousands of sterile workers, a few hundred drones and a single fertile female, the queen. The queen affects the workers in several ways^{3,4}. The presence or absence of the queen is detected by the workers in a matter of minutes. The presence of the queen normally prevents the workers from rearing new queens. Also, the presence of the queen and her brood inhibits the development of the worker's ovaries; in the event of the death of the queen, however, workers do develop their ovaries and lay small numbers of unfertilized haploid eggs. The queen is always surrounded by an ever-changing retinue of about 10 workers at a time who feed and lick her. Most or all these effects of the queen on the workers are mediated by pheromones secreted largely from a pair of mandibular glands on either side of her head. Pheromones are chemicals, usually but not always volatile, secreted from exocrine glands of animals which serve to elicit behavioural or physiological responses in conspecifics and thus serve as chemical messengers⁵. The best-known pheromones such as bombykol, the sex pheromone of the silk moth that helps the male find his mate with incredible sensitivity, are seen to release specific and instantaneous behavioural responses and hence are termed *releaser* pheromones. The honey bee queen pheromone on the other hand, has more complex and fundamental effects on workers, including inhibition of their ovarian development and is hence termed a *primer* pheromone. The queen bee pheromone (which acts both as a releaser and primer pheromone)⁶ is a blend of several chemicals not all of which may yet be known.

However Mark L. Winston and Keith N. Slessor of Simon Fraser University in British Columbia, Canada, have succeeded in identifying five of the most essential components of the queen pheromone which together elicit most of the important behavioural responses expected from the workers⁷. One queen equivalent of what I will call the Winston-Slessor blend (although they prefer to call it QMP, for queen mandibular pheromone, to emphasize their team work involving a large number of other people), consists of about 200 µg of 9-keto-(E)-2-decenoic acid (9-ODA), about 80 µg of 9-hydroxy-(E)-2-decenoic acid (9-HDA), of which about 56 µg is the (–) optical isomer and about 24 µg of the (+) optical isomer, about 20 µg of methyl *p*-hydroxybenzoate (HOB) and about 2 µg of 4-hydroxy-3-methoxyphenylethanol (HVA) (Figure 1). The latter two aromatic compounds are minor and indeed, somewhat unexpected components. On the other hand the aliphatic 9-ODA and 9-HDA are the major components whose involvement in the honey bee queen pheromone has been known for a long time.

The Winston-Slessor blend

The Winston-Slessor blend elicits a clear-cut retinue response, indeed that is how it all started when Kaminiski, Slessor and Winston⁸ noticed that stray worker bees formed a retinue around a glass vial containing a crude extract of the queen mandibular gland. Much of their subsequent work is based on a bioassay based on the retinue response that workers so readily show to the chemicals *sans* the queen bee. The blend also mimics the queen's ability to inhibit queen rearing by the workers. In the event of the death of the queen, workers resort to emergency queen rearing by enlarging some of the cells containing young (< 3 days old) larvae and feeding the chosen larvae with 'royal jelly' and thus channeling them into a developmental pathway leading to the formation of queens. When workers in queen-less colonies were given one queen equivalent of the blend per day, emergency queen rearing was almost completely inhibited. Although the blend elicited other expected responses such as inhibition of swarming, it did not inhibit worker ovarian development, which is therefore thought to be a function of a different primer pheromone not included in the Winston-Slessor blend (and of the brood). An unexpected effect of the blend was its stimulation of pollen foraging and brood rearing. By spraying crops with the blend and showing that more bees visit the crops and whose yield then increases due to better pollination, Winston and his colleagues have demonstrated a promising commercial application of their basic research – what a sigh of relief in this day when there is so much pressure to make economic sense of all scientific research!

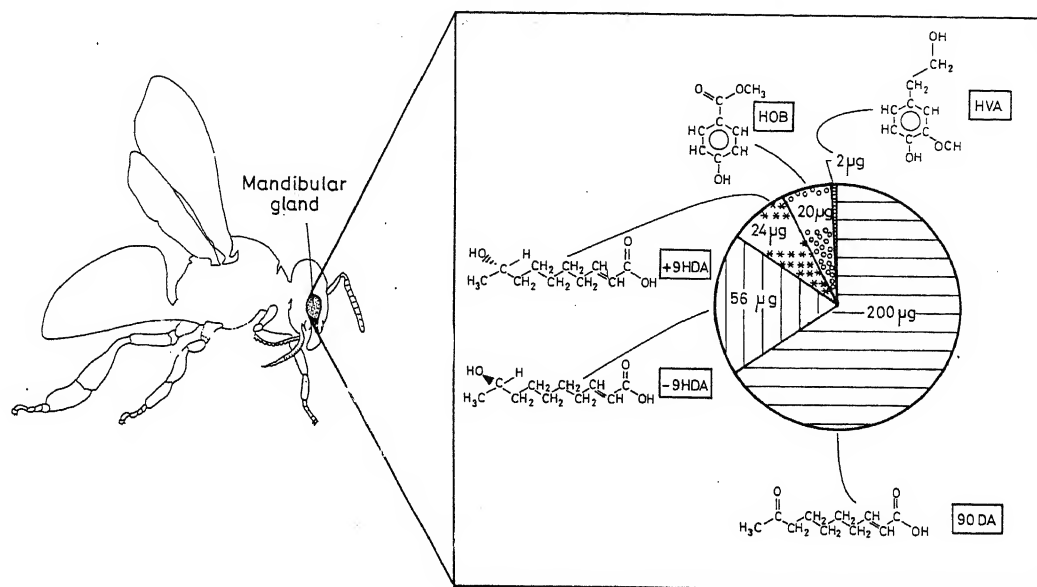


Figure 1. The Winston-Slessor blend of honeybee queen pheromone containing well-defined amounts of five of the most important components of the mandibular gland secretion, which elicits most of the responses expected from workers. See text for expansion of the names of the components.

Queen-worker dichotomy

Having made some economic sense, we deserve the freedom to turn our attention once again to a whole new intellectually challenging question. Perhaps the most fascinating aspect of honey bee colonies is the differentiation of the bees into a sterile worker caste and a fertile queen caste. The question that stems from this observation relates to the possible differences between queens and workers in their pheromone blends and the mechanism of the origin of these differences. These are the questions that Plettner *et al.*⁹ address in a recent path-breaking paper. Workers too produce mandibular gonad secretions that are added to the brood food and may serve as preservatives and nutrients. Instead of the two major components of the queen's secretions namely, 9-ODA and 9-HDA, workers secrete acids hydroxylated at the 10th or ω -carbon atom rather than the 9th or $\omega-1$ carbon atom as in the case of the queen's acids. Instead of the queen's 9-HDA, workers secrete 10-hydroxy-(E)2-decenoic acid (10-HDA) and instead of the queen's 9-ODA, workers secrete the diacid derived from their 10-HDA. In other words, queens and workers differ essentially only in the position of the carbon atom that is hydroxylated. But how does this difference arise? As a result of a series of experiments involving analysis of the fate of deuterated test compounds applied to excised queen and worker mandibular glands, using gas chromatography-mass spectrometry (GC-MS), Plettner *et al.*⁹ have proposed the following caste-specific, bifurcated three step biosynthetic pathway for the production of these compounds (Figure 2).

A caste-specific pheromone biosynthetic pathway

The starting point is stearic acid, a very common, 18 carbon, straight chain saturated intermediate of lipid oxidation¹⁰. In the first step of the proposed pheromone biosynthetic pathway, functionalization is achieved by the addition of a hydroxyl group on either the 18th (ω) or the 17th ($\omega-1$) carbon atom. This functionalization which foreshadows the queen-worker differences depending on whether it happens at the ω or the $\omega-1$ carbon atom is, however, itself not caste-specific; both ω and $\omega-1$ functionalizations occur in both castes to about the same extent. In the second step, the 18-carbon hydroxy acids are shortened to give 10-HDA and 9-HDA by the standard chain-shortening cycles of β oxidation that normally occur during fatty acid metabolism. It is the β oxidation step that is caste-specific – queens preferentially channel the $\omega-1$ compounds and workers preferentially channel the ω compounds into the β oxidation pathway. In the final step, oxidation of the ω or $\omega-1$ hydroxy group that was added in the first step, results in the formation of the diacid in the case of workers and the keto acid in the case of queens. The evidence for every feature of the proposed pathway is clear and convincing. Labelled stearic acid is incorporated into the final products but labelled palmitic and decanoic acids are not. There is no isomerization between 10-HDA and 9-HDA. Both the ω and $\omega-1$ functionalized hydroxy acids are detected to the same extent

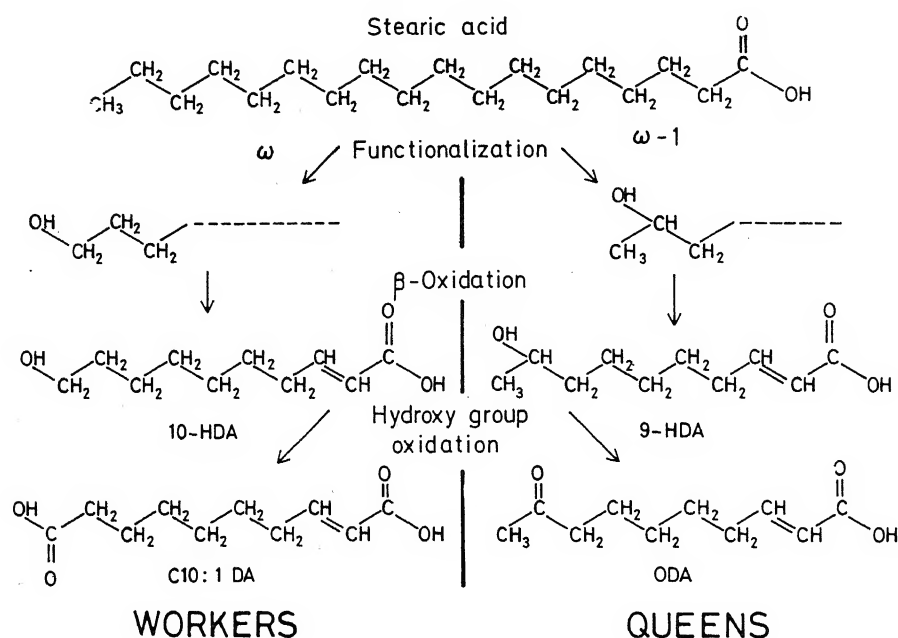


Figure 2. The caste-specific, three-step, bifurcated pathway for the biosynthesis of queen and worker pheromones, as proposed by Plettner *et al.*⁹

in both castes but the subsequent steps are entirely caste-specific. That functionalization precedes β oxidation is evident from the fact that hydroxy acids with more than 10 carbons accumulate when an inhibitor of β oxidation, 2-fluorostearic acid is added to the reaction mixture. Thus both workers and queens add hydroxy groups to either the ω or the $\omega-1$ carbon atom but workers then preferentially convert the ω functionalized compound to produce 10-HDA and the corresponding diacid while the queens preferentially convert the $\omega-1$ functionalized compound to produce 9-HDA and the corresponding keto acid, 9-ODA. Since 9-ODA is the major component of the queen pheromone, I am tempted to dramatize and say that the essence of royalty is just one keto group!

Plettner *et al.*, however, merely conclude modestly from these remarkable findings that 'These results demonstrate how, in a social insect, caste-determined biosynthesis of isomeric compounds can produce markedly different glandular blends that are responsible for many functional differences between queens and workers.' In addition to the dramatization of the essence of royalty as one keto group, I believe that these results permit two other speculations of the considerable significance for our understanding of social evolution.

A chicken and egg problem

The first speculation concerns the usual chicken and egg problem – who came first, the queen or the worker? On the one hand, queens in social insect species can be thought of as being comparable to the undifferentiated

(into queen or worker) adult insects in their solitary ancestors (or, equivalently, in other extant solitary taxa) and the workers can be thought of as being a new invention of sociality. After all, adults in solitary species are all potentially capable of reproducing and it is the character of being sterile and merely working to rear another individual's brood, that is a novel feature of social insects. On the other hand, workers in social species may be thought of as being comparable to their solitary ancestors or extant solitary counterparts and the queens can be thought of as an invention of sociality. After all, adults in solitary species are all capable of nest building, foraging and brood rearing and it is the character of inhibiting reproduction of conspecifics and attempting to become the sole reproductive in a group, at the cost of losing foraging and brood-rearing abilities altogether if necessary, that is a novel feature of social insects. A reasonable solution to this conundrum is to compromise and think of the solitary insects as queen and worker combined because each individual is capable of reproduction as well as nest building, foraging and brood care. And this is a largely correct solution because both queens and workers, at least in the advanced social species, are considerably modified and exaggerated in their respective roles compared to solitary insects. Nevertheless, I believe that, if and when possible, we should try to make an objective assessment of whether queens are ancestral and workers are derived or whether workers are ancestral and queens are derived. I will argue that the pheromone biosynthetic pathway elucidated by Plettner *et al.*⁹, provide one such opportunity.

I hypothesize that the pheromone biosynthetic pathway employed by the workers deviates relatively little from the typical lipid metabolism pathway and is perhaps simply adopted from there. The diacid they make can relatively easily be channeled into an energy-generating role and its degradation products can be profitably fed into the Krebs's cycle. On the other hand, I speculate that the pheromone biosynthetic pathway of the queens is quite a deviation from the standard lipid metabolism pathway. In particular the keto acid is not something one would expect if energy generation is their goal. The expense involved in further breaking down the keto acid makes it a poor candidate to be fed into the Krebs's cycle. I therefore speculate that in the course of making their pheromones, the workers are doing more or less what any solitary insect would do anyway for generating energy from lipids and that their pheromone biosynthetic pathway is therefore the more ancestral one. Conversely, queens have considerably modified the ancestral lipid metabolism pathway in order to make a pheromone that has only lately (relatively speaking) become necessary. In order to do so, they are prepared to make an end product such as the keto acid which is energetically unwise but I argue that energy generation is not their motivation here. Surely they have other mechanisms of generating energy even from lipids. Even if their overall efficiency of generating energy from lipids is lower than that of workers, it does not matter that much because it is the foragers, not the queens, that have to fly great distances in search of food. The pheromone biosynthetic pathway of the queens appears therefore to be relatively more derived. One might also argue that the function of the worker pheromone namely, to act as a preservative and nutrient is also a more ancestral function, more likely to have been useful in the solitary condition. Conversely the function of the queen pheromone appears to be more derived as it fulfills a relatively more recent requirement and hence is unlikely to have been of much use in the ancestral solitary condition. Workers thus seem to use an ancestral biochemical pathway to make a product that may also have been required in the ancestral condition. And queens seem to be using a rather derived form of the biochemical pathway to make a product that has a rather derived function. At least in this limited context, workers seem to be ancestral and queens seem to be derived. This one context, important as it is, cannot be thought to have solved our general problem of who came first, the queen or the worker. It would be prudent, even necessary, to be on the look out for more opportunities to classify queens and workers as ancestral or derived. Indeed, a new and highly derived function of the worker pheromone may yet be discovered which may alter our conclusion. Thus, we may well come up with different conclusions each time and only the relative scores for 'ancestral' and 'derived' that queens and workers accu-

mulate in the long run can help us solve this conundrum in any general sense. But I believe this is a good beginning.

The evolution of caste polymorphism

Yet another striking feature of the social insects, the highly social insects in particular, is the morphological differentiation of queens and workers which may sometimes reach such proportions that, if encountered separately, queens and workers may get classified as different species¹¹. The greatest intra-specific size variations have been recorded in the Asian ant *Pheidologeton diversus*, where some workers weigh 500 times and have a head width 10 times compared to other workers¹². Here the differentiation is not between queens and workers but between the so-called major workers and minor workers. Whether it is between queens and workers or between major and minor workers, these extreme degrees of intra-species, intra-sexual dimorphism require an explanation. The fact that no solitary species seem to match these levels of differentiation suggest that the explanation is linked to the social habit of these insects.

I have recently offered a speculation¹³⁻¹⁵ which was inspired by the idea of evolution by gene duplication first suggested by Haldane¹⁶ and Muller¹⁷ and elaborated and championed by Susumu Ohno¹⁸. The idea is that redundant, duplicate copies of genes can accumulate potentially lethal mutations without killing the organism and eventually can give rise to novel genes coding for novel structures via pathways that would be inaccessible to an individual with a single copy of the gene. I have argued that a very similar consequence will accrue to social insects although for a somewhat different reason. The evolution of altruistic sterile worker castes in the social insects was considered paradoxical until Hamilton proposed the theory of inclusive fitness¹⁹. Today it is common practice to recognize inclusive fitness as having two components, a direct component gained through production of offspring and an indirect component, gained through aiding close genetic relatives. Sterile worker castes are expected to gain fitness exclusively through the indirect component^{4,20,21} and in no other group is there a comparable level of dependence on the indirect component of inclusive fitness.

When some individuals in a species begin to rely on the indirect component of inclusive fitness while others continue to rely on the direct component, as workers and queens in social insects are expected to do, I have argued that different sets of genes in queens and workers will be liberated from previous epistatic constraints and become free to evolve in new directions, because the same individual no longer has to optimize both reproductive and non-reproductive functions. There is no gene duplication here in the conventional sense but the

consequence namely, liberation from previously existing constraints (due to the action of stabilizing selection) and the opportunity to diversify in different directions (through the action of directional selection), is similar. To put it simply, an individual can evolve into a 'super' egg layer if it does not also have to simultaneously be a good forager or it can evolve into a 'super' forager if it does not also have to simultaneously be a good egg layer.

I wish to speculate now that compared to solitary species, social insects are also in a better position to exploit the evolutionary advantages of conventional gene duplication. I have argued in the previous section that the function of the worker pheromone and the biochemical pathway involved in its production are relatively more ancestral and that the function of the queen pheromone and the biochemical pathway involved in its production are relatively more derived. If this is true, it is not difficult to see the tremendous advantage of conventional gene duplication in bringing about the derived condition from the ancestral one. It seems likely that the enzymes involved in the β oxidation step (Figure 2) give rise to specificity for substrates hydroxylated at the ω or $\omega-1$ positions. Imagine that the ancestor of the social insect species had a gene that coded for an enzyme which could deal only with the substrate that was hydroxylated at the ω position. The workers in the descendant social species can continue to use this gene and this enzyme to make worker pheromones which may perhaps have even been made by the ancestor. A duplication of the gene involved can permit the evolution of an alternate enzyme which can handle the substrate hydroxylated at the $\omega-1$ position. We know that such a substrate must already have been available because both kinds of hydroxylations occur to an equal extent in both queens and workers. The duplicated gene would now be free to evolve in new directions without reduced fitness due to the reduction in the efficiency of energy production through lipid metabolism. And new directional evolution can sometimes give rise to substances with such remarkable properties as those of the queen pheromone. A similar chance occurrence of such evolution could hardly have been utilized effectively by a solitary species. Because social insects set aside some individuals for the sole purpose of monopolizing reproduction and inhibiting and controlling all others, they are in a special position to exploit such a consequence of conventional gene duplication.

Evolutionary biologists have often found it useful to clearly distinguish between proximate physiological explanations and ultimate evolutionary explanations. Indeed, failure to make this distinction has sometimes led to unnecessary confusion as to what constitutes a valid answer to the question of why an animal does what it does. However it would be unfortunate if we permanently

delink the study of proximate mechanisms and ultimate evolutionary explanations²². The biochemical pathway for the caste-specific biosynthesis of pheromones elucidated by Plettner *et al.*⁹, constitutes an excellent illustration of how our understanding of the proximate and ultimate factors can mutually reinforce each other. It is high time, that evolutionary biologists became biochemists and vice versa!

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Realtime kinetic analysis of antigen–antibody interaction using solid phase binding: Transformation of hCG-monoclonal antibody complex

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Kinetic constants of MAb–hCG interactions have been determined using solid phase binding of $^{125}\text{I}[\text{hCG}]$ to immobilized MAb. While association has been shown to follow the expected pattern, dissociation consists of at least two reversible steps, one with a rate constant of 0.0025 min^{-1} , and a second with a rate constant of 0.00023 min^{-1} . Validity of affinity constant measurements in the light of the complex reaction kinetics is discussed. A comparison between the method of surface plasmon resonance technology (BIAcore) and solid phase binding (SPB) for determination of kinetic parameters shows that SPB provides not only a cost-effective approach for determination of realtime kinetic parameters of macromolecular ligand–ligate interaction but also a method with several advantages over the BIAcore system in investigating the mechanism of antigen–antibody interaction.

ANALYSIS of ligand–ligate interaction has found renewed surge of interest with the development of surface plasmon resonance technology to study realtime kinetics of the interaction with BIAcore^{1–5}. Using this approach kinetic constants have been determined for several ligand–ligate pair, most common being MAb–antigen pair. Several kinetic and thermodynamic constants obtainable by the BIAcore can also be obtained, in theory, by radiolabelled ligand binding to MAbs in conventional approach like liquid phase RIA (LPRIA), enzyme-linked immunosorbent assay (ELISA), etc. However this simple approach has not been seriously attempted in the past, probably for the following reasons. First, extensive use of ELISA, in most of the MAb-related research, was the method of choice for study. However attempts to use ELISA for determination of even simple equilibrium constant were inconsistent^{6–12}. Other alternate method, namely LPRIA with MAbs is cumbersome for kinetic studies with radiolabel being an added deterrent. In addition, as has been shown by us recently, both the methods LPRIA & ELISA are multistep processes and suffer from post-equilibrium disturbance, due to easy dissociability of the MAb–Ag complex^{13,14}. Develop-

ment of single step solid phase assay using immunochemically adsorbed antibodies allows instantaneous termination of the reaction at equilibrium, and hence, eliminates uncertainties that exists in ELISA/LPRIA¹⁵. Thus binding of radiolabelled antigen to immobilized MAbs provides a method by which kinetic analysis of antigen–antibody interaction can be investigated. In the following we determine the kinetic constants of the reaction and analyse dissociation pattern of MAb– $^{125}\text{I}[\text{hCG}]$ complex and discuss results in the light of BIAcore analysis.

Materials and methods

Human chorionic gonadotropin (hCG) was prepared from early pregnancy urine and characterized as reported earlier¹⁶. Iodination grade hCG was obtained from NHPP, Bethesda, USA. MAb VM4 was raised in our laboratory by a standard procedure using hCG $\alpha\beta$ dimer for immunization. This MAb was found to be specific for the native β subunit and reacted well with the dimer.

Iodination of hCG was carried out by iodogen method¹⁷. Specific activity was calculated by the extent of incorporation. Unless otherwise stated, the specific activity was 45,000 cpm/ng hormone. Freshly prepared $^{125}\text{I}[\text{hCG}]$ was used in all kinetic experiments.

Scatchard plot analysis

MAb was immobilized through immunochemical bridge on microtiter wells as already described^{13–15}. MAb VM4 culture fluid was coated at a dilution of 1/1000 for SPRIA. SPRIA was carried out by the standard procedure and the data was subjected to Scatchard analysis to obtain apparent affinity constant¹⁸ (K_a).

Determination of association constant

$^{125}\text{I}[\text{hCG}]$ was added to VM4-coated wells (coated with 250 μl of 1/50 VM4 culture fluid) and binding was ter-

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minated at different periods of time by washing the wells with RIA buffer. Radioactivity bound to the wells was monitored and used for determination of the rate constant by the following formula.

$$k_{+1} = [dx/dt]/[Ag] \cdot [MAB],$$

where dx/dt represents the slope of the binding data, $[Ag]$ and $[MAB]$ represent the initial concentrations of antigen and antibody, all expressed in moles/litre.

Determination of dissociation rate constant of hCG-VM4 complex

$^{125}\text{I}[\text{hCG}]$ was bound to immobilized MAb overnight, washed and counted in a multigamma counter. Dissociation was started immediately by adding 250 μl of hCG (2 $\mu\text{g}/\text{ml}$) in 0.05 M phosphate buffer, pH 7.0

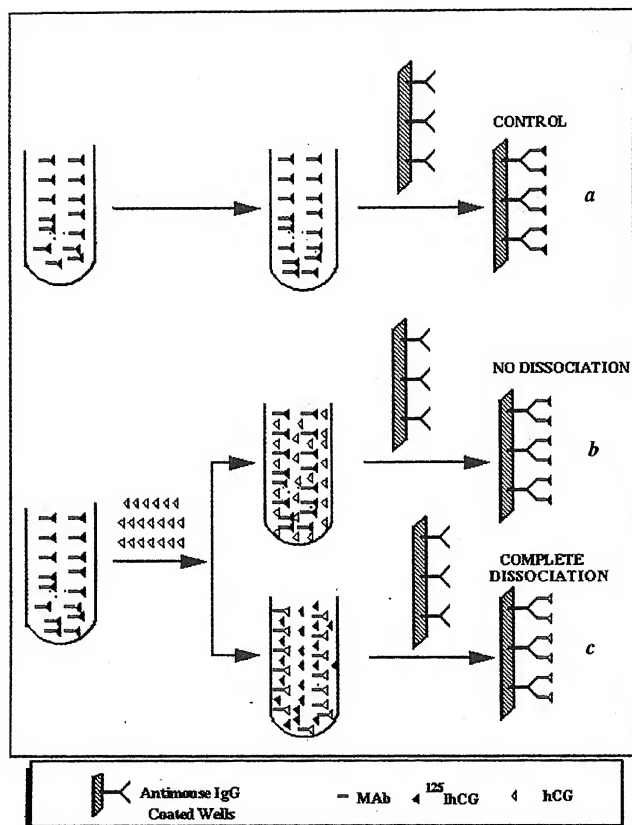


Figure 1 a-c. Schematic representation of the dissociability of $^{125}\text{I}[\text{hCG}]$ -VM4 complex in liquid phase (a) control. (b) and (c) show the situation when the $^{125}\text{I}[\text{hCG}]$ -MAb complex is completely non-dissociable or completely dissociable. In a completely dissociable situation, the binding of the complex formed in the liquid phase to the Mouse DAb coated well will be nonspecific (c), while in the case of complete nonreversibility it will be like specific binding (b) as in the control (a). Intermediate values present partial reversibility (See methods for experimental details).

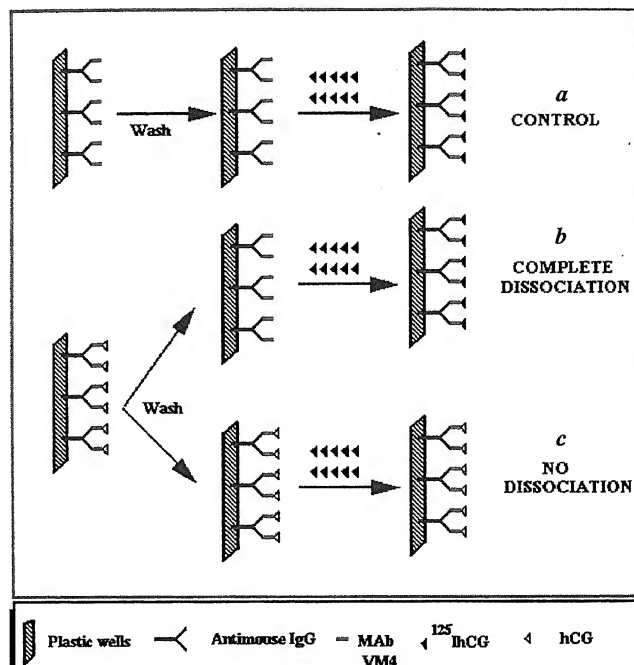


Figure 2 a-c. Schematic representation of the dissociability of hCG-VM4 complex from the solid phase (a), binding of $^{125}\text{I}[\text{hCG}]$ to VM4 coated well (control). (b) and (c) show sequence of events when the VM4-hCG complex dissociates completely or does not dissociate. Complete dissociation results in binding of $^{125}\text{I}[\text{hCG}]$ comparable to control (a) and lack of dissociation results in nonspecific binding (c). Intermediate values present partial dissociation (See methods for experimental details).

containing 2% bovine serum (RIA buffer). At known intervals of time, the supernatant was discarded and the radioactivity bound was measured in the same carrier of the multigamma counter as done before (this is to take care of different counting efficiencies of the carriers). Radioactivity measured at each point of time is expressed as percentage of the radioactivity retained by the well¹⁹. For determining the dissociation rate constants plot of $\ln[(a-b)/(x-b)]$ vs time was drawn from the data. Value of a was 90%, while that of b was 48% for fast dissociation rate constant (x was taken between 0 and 400 min for the fast dissociation), while for the slow dissociation they were 48% and 0% respectively (x values taken for 13 h, 24 h and 48 h: see under results for details).

Capacity measurement of the well

Capacity of the well to bind hCG was measured by specific binding, specific activity of the $^{125}\text{I}[\text{hCG}]$ and K_a determined by Scatchard plot using the formula

$$C_{\text{MAb}} = [B/F + K_a \cdot B] \cdot [1/K_a],$$

where B and F represent bound and free antigen concentrations, C_{MAB} represents the capacity of the well and K_a affinity constant. This method is routinely used for determination of the concentration of MAb required for rate constant measurements.

Determination of apparent nonreversibility

In liquid phase. The rationale of the approach is shown in Figure 1. Experimentally, $^{125}\text{I}[\text{hCG}]$ (200,000 cpm) was incubated with VM4 antisera in the liquid phase in a tube for 1–2 h at room temperature. 100 μl of the $^{125}\text{I}[\text{hCG}]$ -VM4 complex (100,000 cpm) is added to immobilized antimouse IgG (in microtiter wells) and binding carried out for 2–4 h at room temperature. As control, the same amount of $^{125}\text{I}[\text{hCG}]$ (100,000 cpm) incubated in RIA buffer was added. Radioactivity bound to the wells is a measure of the extent of nonreversibility.

In solid phase. Figure 2 schematically represents the methodology of the experiment (see legend for details). Immobilized VM4 on plastic wells was saturated with hCG by incubating with 2 $\mu\text{g}/\text{ml}$ hCG for 20 h. Unbound hCG was washed off and hCG bound to the antibody was incubated with RIA buffer (250 μl) for 2 h to allow dissociation to occur. The wells were washed, further dissociation allowed to occur for an additional ten times. Finally $^{125}\text{I}[\text{hCG}]$ (100,000 cpm, 250 μl) was added to the well and after 20 h incubation period the radioactivity bound was measured in a LKB multigamma counter. As control VM4 adsorbed wells were also washed with RIA buffer the same number of times, except they were not saturated with hCG. Difference between the specific binding of these two sets gives an index of the extent of nonreversibility.

Determination of affinity constant

By Scatchard plot. Affinity constant was obtained by the standard method already described.

From re-equilibrium data. $^{125}\text{I}[\text{hCG}]$ (250 μl , 100,000 cpm) was added to immobilized VM4 and specific binding after 20 h incubation was determined (S). To each of the wells was added 250 μl of RIA buffer and re-equilibration of the $^{125}\text{I}[\text{hCG}]$ allowed to occur between the solid phase and solution phase for 20 h at room temperature, supernatant discarded and radioactivity bound was measured (B). Another set of wells were incubated overnight with 250 μl of 2 $\mu\text{g}/\text{ml}$ hCG. Radioactivity that remained bound to this was taken as 'apparently nondissociable' $^{125}\text{I}[\text{hCG}]$ from the complex (N). From this data K_a was calculated by the formula

$$K_a = [B - N] / [S - B] \cdot [(C_{\text{MAB}} - N) - (S - B)],$$

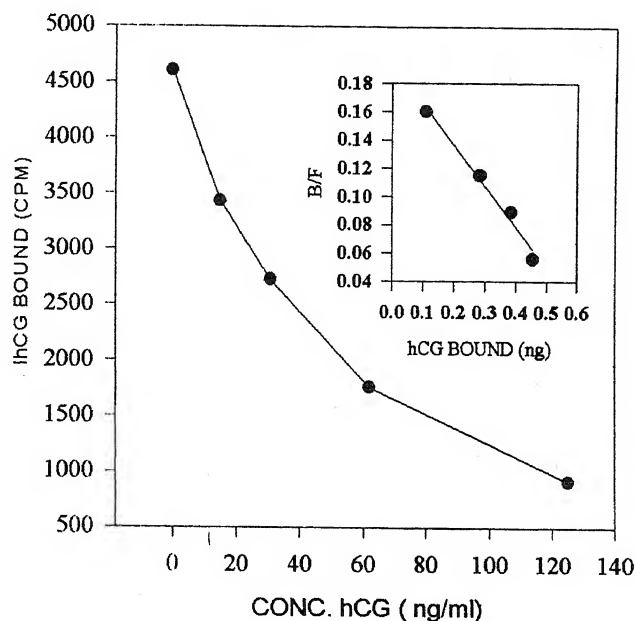


Figure 3. SPRIA and K_a determination by Scatchard and single point binding. Inset shows Scatchard plot for the displacement analysis.

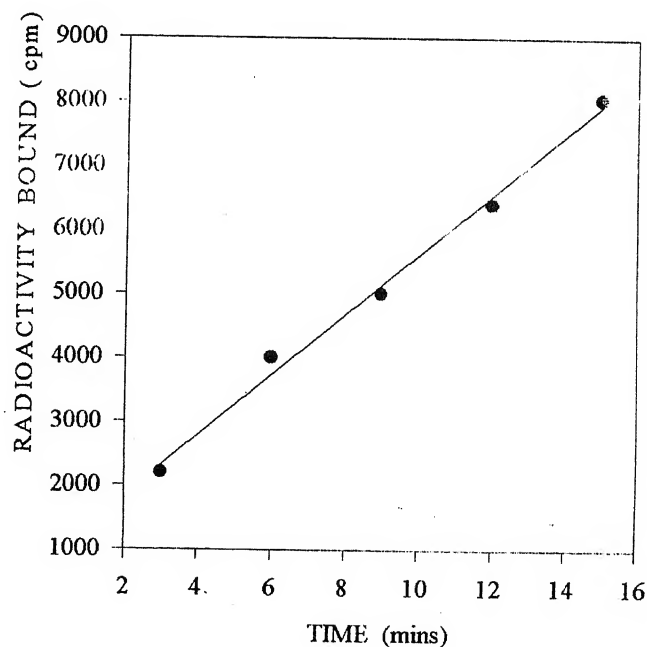


Figure 4. Determination of k_{+1} by binding data: $^{125}\text{I}[\text{hCG}] = 112,000 \text{ cpm/well}$ of specific activity $26,000 \text{ cpm/ng}$ added to VM4 coated wells at 0 time. Capacity of the MAb adsorbed calculated from overnight binding was $5.193 \times 10^{-10} \text{ M}$ and that of $^{125}\text{I}[\text{hCG}]$ $4.657 \times 10^{-10} \text{ M}$ respectively (see for details under methods). Affinity constant taken for calculation is $3 \times 10^9/\text{M}$, obtained from the data of Figure 3.

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where all values are expressed in concentrations (moles/litre). C_{Mab} is the concentration of the MAb immobilized on the well, obtained from overnight binding data as explained earlier.

From rate constants. Forward rate constant (k_{+1}) and reverse rate constant/s (k_{-1}) determined as described above have been used to derive K_a by the formula

$$K_a = k_{+1}/k_{-1}.$$

Results

Scatchard plot analysis of SPRIA using immobilized VM4 for the determination of apparent affinity constant gave a value of $3 \times 10^9 \text{ M}^{-1}$ (Figure 3). Affinity constant measured by single well binding also gives comparable results¹⁹. Hence in all further experiments concentration of bound MAb is calculated from single point binding data as explained under methods.

Binding of $^{125}\text{I}[\text{hCG}]$ to immobilized VM4 with time is linear (Figure 4). Rate constant of association measured using the slope of this plot is $8 \times 10^6 \text{ M min}^{-1}$. This is comparable to that obtained in several MAb–Ag pair using the BIAcore method^{20–22}.

In contrast to the association profile which follows a linear pattern, dissociation profile of the $^{125}\text{I}[\text{hCG}]$ –

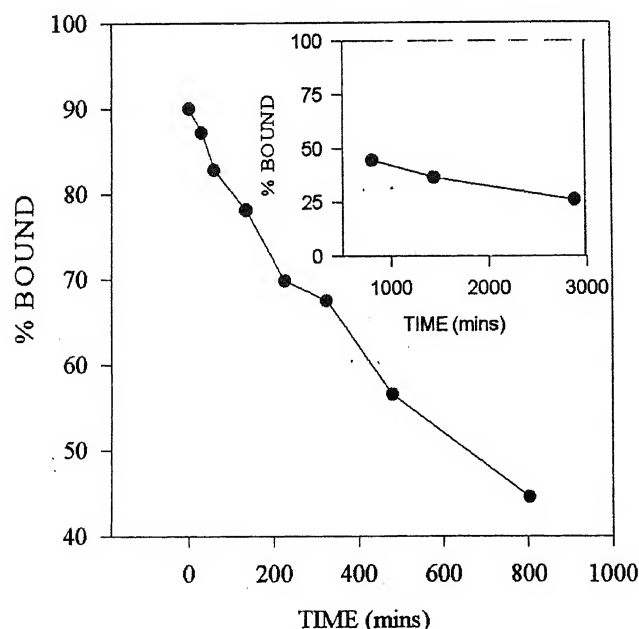


Figure 5. Dissociation profile of bound $^{125}\text{I}[\text{hCG}]$ –Mab complex: $^{125}\text{I}[\text{hCG}]$ (86,000 cpm of specific activity 42,000 cpm/ng, 250 μl) was added to microtiter wells previously coated with Mab VM4 at 1/100 dilution for overnight binding (cpm bound = 35876 ± 2918), and dissociation started by adding 250 μl of unlabelled hCG (200 ng/well). Inset shows the extent of retention of binding for extended periods of incubation (13 h to 48 h).

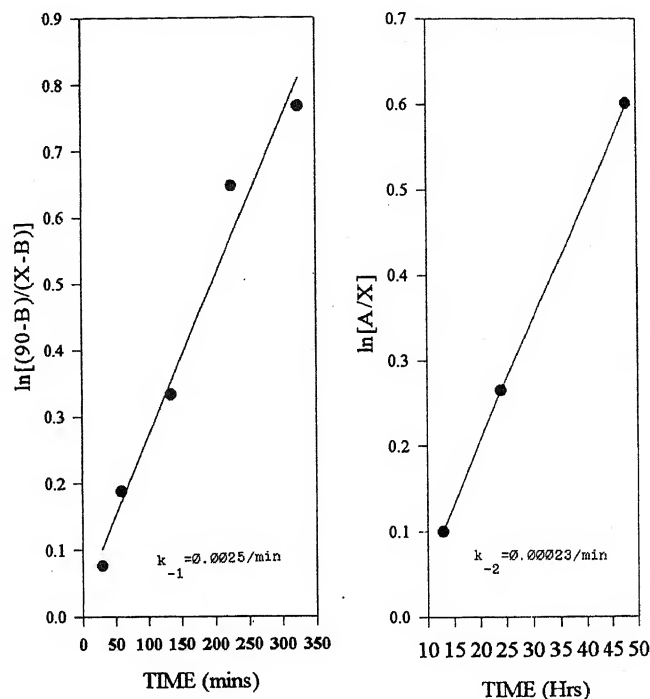


Figure 6. Plot for determination of dissociation rate constant. Extrapolated binding to 0 time is considered (52%) as maximum dissociable for the determination of the fast rate of dissociation ($a = 90$, $b = 48$). Right panel shows the 'dissociation rate constant' obtained for the slow dissociation with $a = 52$, and $b = 0$ (See under methods for details).

Table 1. Dissociation of the $^{125}\text{I}[\text{hCG}]$ –VM4 complex in liquid phase binding ($^{125}\text{I}[\text{hCG}]$ bound to VM4 coated wells in cpm)

VM4 dilution used during complex prepn	Non sp.* control	Exptal	Sp. control	% non-reversible
1/400	1845	12,300	33,212	37
1/800	1300	12,500	28,500	43
1/1600	908	8,150	19,800	40
1/3200	520	4,700	12,800	37

*This control presents the $^{125}\text{I}[\text{hCG}]$ incubated with VM4 in the presence of 2 $\mu\text{g/ml}$ hCG. Exptal represents $^{125}\text{I}[\text{hCG}]$ incubated with VM4 in absence of unlabelled hCG (overnight) to which 2 $\mu\text{g/ml}$ hCG was added and reequilibrated (120 min) before adding to immobilized Dab. Sp. controls represent binding of $^{125}\text{I}[\text{hCG}]$ VM4 at respective concentrations to immobilized Dab (Antimouse IgG).

Mab is more complex. Even after 8 h of incubation with excess unlabelled hCG complete dissociation of the complex does not occur (Figure 5) with further dissociation occurring rather slow but certain (inset), indicating two distinct rates of dissociation, and the two rate constants of dissociation are 0.0025 min^{-1} and 0.00023 min^{-1} respectively (Figure 6). The first rate constant (k_{-1}) is obtained by assuming the total dissociable $^{125}\text{I}[\text{hCG}]$ to be 52% based on the extrapolated

Table 2. Dissociability of hCG-VM4 in solid phase

	Binding of radioactivity to immobilized VM4 (<i>n</i> = 7) cpm \pm SD
Control [Binding of ^{125}I [hCG] to immobilized VM4 well]	50,400 \pm 3650
Experimental [Binding of ^{125}I [hCG] to immobilized VM4, saturated with hCG and extensively washed for complete dissociation]	33,519 \pm 3000

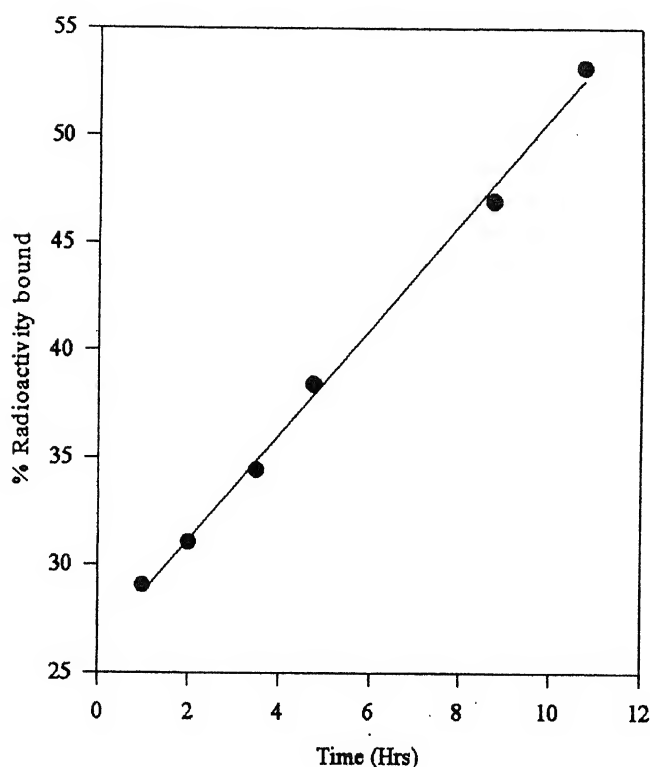


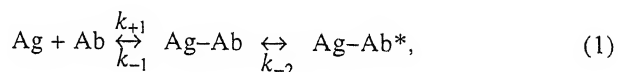
Figure 7. Increase of slowly dissociable portion (transformed complex) of preformed complex of VM4- ^{125}I [hCG]. Radioactivity bound at 1 h incubation period (0 time of dissociation) was 16,450 \pm 540 (SD) cpm. Unbound ^{125}I hCG was discarded and dissociation started at time periods as indicated in X-axis.

slow dissociation data (curve in the inset of Figure 5 extrapolated to 0 time) while the second rate constant (k_{-2}) is obtained by assuming that the slow dissociation is complete in due course, and analysing the points above 8 h by which time most of the fast dissociation is complete.

Dissociation observed in the above solid phase method is not an artifact arising out of iodination of hCG or because of solid phase binding has been proved by demonstrating that reaction of the MAb with ^{125}I [hCG] in liquid phase is as much 'nondissociable' as

in the solid phase (Table 1). The extent of slow dissociability seen is about 40%, comparable to that obtained in SPB (35%). Similarly VM4 saturated with unlabelled hCG in the solid phase does not completely dissociate in spite of a dozen washings (Table 2) and rules out slow dissociation as an artifact arising out of labelled hCG. Apparent nondissociable portion of the binding increases linearly with time (Figure 7) in absence of either of the reactants. This increase is quite significant going up to 50% in 8 h. However incubation of this complex with unlabelled hCG slowly releases the ^{125}I [hCG] into medium, indicating this apparent nondissociation to be infact a slow dissociation. The result also indicates that this transformation appears to be spontaneous, as it occurs in the absence of any other reactants.

On the basis of these kinetic evidences, the mechanism of MAb-hCG interaction can be presented as follows to account for the rate constants.



where Ag-Ab and Ag-Ab* present dissociable and transformed complex respectively. Conversion (apparent dissociation) rate constant of Ag-Ab* to Ag-Ab is about 10% compared to the dissociation rate of Ag-Ab to Ag and Ab. This proposed mechanism is for MAb VM4, but is likely to be true for other epitope-paratope pairs. Relevance of this slowly transformed Ag-Ab* in physiology also needs to be studied further. Apparent nonreversibility of several polyclonal antibodies clearly indicates that such transformed species are present in polyclonal antisera-antigen complexes and hence may have relevance in physiological systems.

On the basis of these studies, there are several ways to obtain affinity constants of VM4- ^{125}I [hCG] interaction. It is certain that K_a as determined by Scatchard plot, though extensively used, may not quantify avidity of the system. A comparison of affinity constants measured for this system using different methods shown in Table 3 demonstrates that the affinity constants measured are not very different from Scatchard plot data. However caution should be exercised in the choice of the disso-

Table 3. Affinity constant measurements by different methods (M^{-1})

Scatchard plot from SPRIA	3.00×10^9
By single well binding	2.97×10^9 (<i>n</i> = 6)
By reequilibration data	6.60×10^9 *
By k_{+1}/k_{-1} (using fast dissociation)	3.33×10^9 **
By k_{+1}/k_{-1} (using slow transformation)	33.30×10^9 ***

*Input ^{125}I [hCG] = 86,000 cpm, bound overnight = 59,000 cpm, nondissociable by 48 h incubation with hCG = 9500 cpm, ^{125}I [hCG] bound after overnight reequilibration in 250 μl of RIA buffer = 29,200 cpm.

**Using k_{+1} of $0.8 \times 10^7 \text{ min}^{-1}$ and k_{-1} of 0.0025 min^{-1} .

***Using k_{+1} of $0.8 \times 10^7 \text{ min}^{-1}$ and k_{-2} of 0.00023 min^{-1} .

ciation rate for the calculation of the K_a , because slow transformation is an integral part of the binding reaction.

Discussion

Reaction kinetics have been investigated in several systems using radioligands. Unsuccessful attempts of the same approach with antigen-antibody interaction can be traced to the error of the analytical tool that was adopted, namely ELISA and LPRIA. Both are multistep assay systems, and during post-equilibration period reversibility of MAb- $^{125}\text{I}[\text{hCG}]$ has been clearly demonstrated to introduce errors in interpretation¹³. Solid phase binding (SPB), a single step method, which eliminates the errors arising out of reversibility has made it possible to investigate the kinetic parameters.

Results presented clearly demonstrate that kinetic constants can be measured using binding of radiolabelled ligand to MAb adsorbed immunochemically to plastic wells. Binding of $^{125}\text{I}[\text{hCG}]$ to immobilized matrix follows a second order reaction as expected with a measurable rate constant which falls in the range of the data generated for several MAb-Ag pairs using the BIAcore system. Contrary to expectations, dissociation in presence of 1000-fold excess of hCG is not complete though it follows a first order reaction with measurable rate constant, and 35% of the radiolabel remains bound to the well even after 20 h of incubation. Also, this apparent nondisplaceability is not artifactual arising out of iodination of the hCG, as nondissociability is seen in solid phase binding of unlabelled hCG (Table 2). In fact immobilized MAb-hCG (saturated with unlabelled hCG) after repeated washing fails to bind $^{125}\text{I}[\text{hCG}]$ to the same extent as free immobilized MAb (Table 2), and clearly demonstrates that partial nonreversibility is indeed true in both solid and liquid phases even when using nonradioactive ligand. Though the extent of nondissociability is 30% with VM4, several antibodies show as much as 50–80% nondissociability, and hence indicate its significance.

Based on the two step pattern of dissociation, a reversible one and the second slow-reversible one, reaction of an MAb with $^{125}\text{I}[\text{hCG}]$ can be presented as in (eq. 1). First step promoted by epitope-paratope interaction leads to a complex (Ag-Ab), which is in reversible equilibrium with free antigen and antibody; second step consists of its transformation to another complex (Ag-Ab*). One of the immediate questions this complex dissociation data projects is the validity of affinity constants extensively employed as a quantitative measure of avidity. Affinity constants measured by different methods tabulated show that it is not significantly different. Scatchard value is similar to that obtained by individual rate constant determinations representing the first step of the reaction. However affinity constants as measured

from re-equilibrium results (Table 3) are also comparable though on the higher side. Considering that in this case the extent of nonreversibility is 30%, K_a measured by all the methods are comparable. If nondissociability were to be 50–80% as it happens in some MAbs, the measured K_a considering the nondissociable portion could be different from that obtained by direct Scatchard measurements/binding data/ $[k_{+1}/k_{-1}]$ measurements. The nondissociability data indicate that caution is needed in assessing K_a by Scatchard plot, and in adopting appropriate methods for relative measurements. An absolute measure of K_a is unlikely to be determined from binding data methods, like Scatchard plot, single well binding, etc. Infact affinity constant needs to be redefined to the requisite step of the reaction.

Analysis of the dissociation data points to transformation of the primary Ag-Ab complex to a transformed (slow-dissociable) Ag-Ab* type (Figures 6, 7). The chemical or physicochemical events which lead to this are not known presently. This is not associated with a covalent binding of MAb-hCG is certain as 5% formic acid completely dissociates the radiolabelled hCG instantaneously (data not shown). How the transformation is related to epitope-paratope interaction is also unclear. Likewise physiological significance of the transformed complex is unknown.

Analysis of the kinetics of dissociation shows that the reaction of an antigen with its antibody is not a simple epitope-paratope reaction and the mechanism proposed here is a minimal requirement. While kinetically the mechanism of the reaction can be presented as shown in eq. (1), additional chemical data is needed to investigate the nature of the reaction. But, it is certain that the interaction of antigen-antibody though promoted by epitope-paratope pair may have other components which are unknown. The regions involved in subsequent conversions are not known, but definitely it is not promoted by the presence of either hCG or Mab (Figure 7).

There are two other methods reported which can measure kinetic constants of MAb-Ag reaction – the BIAcore method which uses surface plasmon resonance^{1-5,20-23}, and the method of Larvor *et al.* using liquid phase reaction coupled to ELISA for determination of dissociation rate constant²⁴. Presently, the former method is used widely because of the ease of determination and the rapidity with which results can be obtained. The method used in this paper (SPB method) is comparable to the BIAcore method in that both are heterogeneous reaction systems where one of the ligands is immobilized. Moreover, association and dissociation constants can be determined by both the methods. Comparisons between these two systems are illustrated in Figure 8. The top panel shows diagrammatically the flow-cell of a BIAcore having dimensions of $50 \times 500 \times 2000 \mu\text{m}$. Flow of solutions is through the

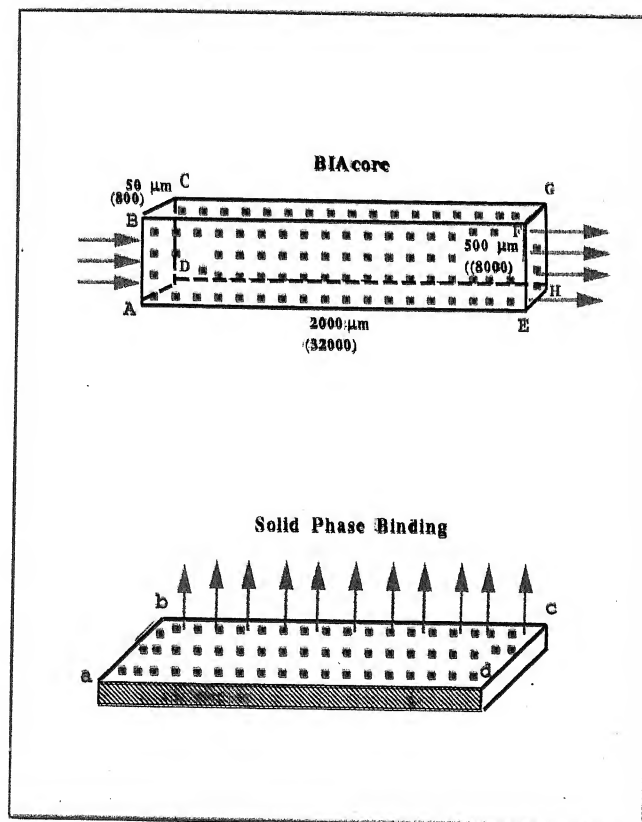


Figure 3. Schematic comparison of the flow cell of BIAcore (top panel) and plastic surface of SPB (lower panel). Arrows show the direction of flow of the solvent in flowcell of BIAcore. ■ presents immobilized ligate covalently bound to the flow cell (in BIAcore) or adsorbed immunochemically (in SPB). Numbers (in μm) represent the dimension of the flow cell, while the corresponding numbers in the brackets represent the number of molecules of the ligate bound along the corresponding axis. Total number of molecules bound in the BIAcore flow cell is about 20×10^{10} molecules. Note that the ligates are stacked up within the flow cell in three dimension, while in the SPB (lower panel) it is distributed in two dimension only.

Table 4. Molecular distance between immobilized antibody molecules in BIAcore cell and in plastic well

Adsorption on	Capacity measured	Surface/volume	Distance between two molecules
Microtiterwells	4 ng/well	81 sq. mm	$6.5 \times 10^{-2} \mu\text{m}$
	1 ng/well	81 sq. mm	$8.0 \times 10^{-2} \mu\text{m}$
BIAcore chip at			
(a) RU of 693	$0.5 \mu\text{M}^*$	60 nl	$14.0 \times 10^{-2} \mu\text{m}$
(b) RU of 10,000	$7.2 \mu\text{M}$	60 nl	$5.5 \times 10^{-2} \mu\text{m}$

*Taken from Hall *et al.*²⁶.

cross-section of ABCD shown by the arrow. The flow-cell has immobilized ligate in space in multilayers illustrated by small squares. Distance between each ligate in

both BIAcore and SPB (bottom panel) is about $6 \times 10^{-2} \mu\text{m}$, almost 10–50 molecular distances apart from each other (Table 4). Thus both the systems have the same molecular disposition of the ligates, and are subject to similar criticisms on these grounds. While immobilization is done through covalent binding in BIAcore, immunochemical bridge has been used in the SPB method. By the nature of the immobilization of ligates each method has its advantages. Two assumptions made in analysis of the results from BIAcore have been that association is not diffusion controlled and dissociation is not complicated by rebinding of the dissociated ligand. Both the assumptions have been questioned^{25,26} and the nature of the problem can be illustrated with Figure 8. In dissociation, which starts with saturated ligand, the solute enters the cells at the cross-section ABCD traverses through and exits at EFGH cross-section. As can be seen in the Figure the dissociated ligand at the cross-section near ABCD has to pass through a very large number of molecules of the immobilized ligate (in this case about 17×10^{10} molecules) which allows rebinding of the dissociated ligand. This error is rather small in early stages of elution (5–10 s by which time about 10 volumes of the solvent has flown through), but tends to become more and more as dissociation increases, effectively distorting the dissociation pattern much like in chromatography and underestimates the true dissociation rate. This effect has been demonstrated by Nieba *et al.*²⁵, and becomes highly significant if the association constant is of the order of 10^5 M s^{-1} , a range common in most MAb–Ag systems^{20–23}. Similarly if the association constant of the ligand is very high [$>10^7 \text{ M s}^{-1}$] binding is limited by diffusion rather than the binding constant. This is especially so because the ligate is embedded in dextran matrix in three dimension and hence diffusion is an obligatory first step in the binding. Methods have been recently suggested to overcome these problems, but the corrections to be adopted require good knowledge of kinetics, and are unlikely to be the same for all the ligand–ligate pair. In contrast to BIAcore, SPB method utilizes the ligate immobilized on a single surface (Figure 8, lower panel) and does not suffer from the disadvantage of reassociation. Since the ligate is fixed as a monolayer, free access of the ligand to the ligate exists, and hence does not impose a limitation based on diffusion as in the BIAcore. Thus these two major problems associated with BIAcore do not exist with the SPB. Complex dissociability pattern, which has not been quantitated so far in the BIAcore, can be quantitated by the SPB method. Apparent nonreversibility present in protein–protein binding is clearly demonstrated in the dissociation patterns in the BIAcore approach as well, but mostly neglected in all interpretations^{20–22}. Quantitation of slow dissociation throws light on the mechanism of ligate–ligand interaction better through kinetic approach. In contrast to these advantages of the SPB

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method, need of radiolabelled ligand is a disadvantage needing pure antigen for investigations and a need to prove that the results obtained are not artifactual arising out of iodination. However the results presented here indicate that they are not artifactual and are likely to be the same for all other ligand–ligates. Quantum of the ligate that can be incorporated into the flowcell of BIAcore is 1000-fold more than in the SPB method, which makes it possible to investigate low affinity binding [$\times 10^5/M$] using BIAcore while the affinity of the ligand–ligate should be high [$\times 10^7/M$] for the SPB method.

Though data presented here are for the antigen–antibody pair, the approach is not limited to only this ligand–ligate pair, or to only radioiodine-labelled antigens. The same approach can be used to study other important ligand–ligate pair like that of protein–RNA, protein–DNA, etc. Demonstration of transformation of reversible Ag–Ab complex to slow-dissociable complex (Ag–Ab* type) indicates the possibility that such as yet unrecognized interactions may also be present in other ligand–ligate pair. Thus the SPB method provides a very cost-effective approach to study the realtime kinetics of ligand–ligate interaction. Such studies may be expected to provide new valuable information on the interaction of macromolecular ligand–ligate pair. Importance of the transformed complexes in physiology/physiological processes needs to be investigated.

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Liquid drops in rise against gravity through a viscous medium: Drag force by the method of dimensions and comparison with liquid drops in fall under gravity

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We have presented previously the results observed in the study of liquid drops of Reynolds number (20–305), Eotvos number (2×10^{-2} to 105×10^{-2}) and Morton number (4.75×10^{-12} to 3.75×10^{-7}), in free fall without exhibiting oscillation through an immiscible liquid column where the liquid in the column is lighter than the liquid drops which tend to fall (downward motion) under the influence of gravity from the top of the liquid column. Suppose the column liquid is heavier, then, obviously, the motion of the liquid drop is completely curtailed and the drop floats. However, the motion of the drop may be achieved by its rise (upward motion) from the bottom of the liquid column. The rise of the drop occurs against (the influence of) gravity. The motion of the drop in rise against gravity may be thought or assumed to be opposite to that of the motion of the drop in fall under gravity. Therefore, we have devoted attention here to study the drops in rise against gravity through an immiscible liquid column to understand whether the expressions satisfying the drop in fall under gravity also hold good for the drop in rise against gravity or not and to determine the proportionality constant K of the drag force expression (eq. (1)) which suits the drop in rise against gravity and another constant S (eq. (6)) just as observed for drops in fall under gravity. In the present study, the drops in rise against gravity considered are in the range of Reynolds number, R_e (25–495), Eotvos number, E_t [(2×10^{-2}) to (70×10^{-2})] and Morton number, M_o [(2.06×10^{-12}) to (7.13×10^{-10})]. The important observations made in the present study are that (i) the value of the proportionality constant of the drag force expression for drop in rise against gravity differs from the value of the proportionality constant of the drag force expression for drop in fall under gravity and (ii) the value of the constant S in the case of drops in rise against gravity differs from the value of the constant S in the case of the drops in fall under gravity¹. For drops in rise against gravity, here, the predicted value for K and S are respectively 4.6082 (≈ 4.6) and 0.3176 (≈ 0.32) $m^{-1} s^2$; for drops in fall under gravity, they were predicted as $K = 3.6094$ (≈ 3.6) and $S = 0.2483$ (≈ 0.25) $m^{-1} s^2$.

RECENTLY Srinivasan and Satyanarayana¹ have presented the results observed in the study of liquid drops in the range of Reynolds number, R_e (20–305) ($R_e = \sigma u D / \eta$), Eotvos number E_t (2×10^{-2} to 105×10^{-2}) ($E_t = g \Delta \rho D^2 / \gamma$), and Morton number, M_o (4.75×10^{-12} to 3.75×10^{-7}) ($M_o = g \eta^4 \Delta \rho / \sigma^2 \gamma^3$), in free fall without exhibiting oscillation through an immiscible liquid column (column liquid is lighter than the drop). $\Delta \rho$ is the absolute value of density difference, $|\rho - \sigma|$. In their study¹, they obtained a fresh expression for the drag force by the method of dimensions for the drop in fall from the top of the liquid column under the influence of gravity. When the liquid drop is lighter, the motion of the drop occurs by rise from the bottom of the column against gravity. Since the motion of the drop in rise against gravity may be thought to be opposite to that of the motion of the drop in fall under gravity, an attempt has been made in this paper to study the rise of liquid drop against gravity without exhibiting oscillation through an immiscible liquid column by the method of dimensions and to arrive at results to know whether they are in line or not with the observations that were already made in the case of liquid drops falling without exhibiting oscillation under the influence of gravity¹ through an immiscible liquid column. Studies on the rise of liquid drops through an immiscible liquid column have been carried out by several authors^{2–28}. Literature review shows that none has attempted to deal with the problem of rise of liquid drops with only six variables F , D , u , η , ρ and σ in the dimensional analysis of the drag force F (F is the drag force acting on the drop in rise; D is the diameter of the drop; u is the terminal velocity attained by the drop; η is the viscosity of the liquid in the column; ρ is the density of the liquid drop; and σ is the density of the liquid in the column). Therefore these six variables F , D , u , η , ρ , σ alone are considered to arrive at an expression for the drag force (eq. (1)) acting upon the drop in rise against gravity without exhibiting oscillation through an immiscible liquid column by the method of dimensions. Since Srinivasan and Satyanarayana¹ have employed only these six variables to arrive at a fresh expression for the drag force acting on the drop in fall under gravity without exhibiting oscillation through an immiscible liquid column, an attempt has also been made to know whether the expressions (eq. (1)–(10)) given by them by the method of dimensions for drops in fall under gravity hold good for the drops here in rise, against gravity or not. Drops of Reynolds number, R_e (25–495), Eotvos number, E_t [from (2×10^{-2}) to (70×10^{-2})] and Morton number, M_o [from (2.06×10^{-12}) to (7.13×10^{-10})], which rise without oscillations against gravity through an immiscible liquid column are dealt with in the present study. The experimental data points predict 4.6082 (≈ 4.6) for the constant K of the drag force expression (eq. (1)) which suits drop in rise against gravity. The predicted value of K is

3.6094 (≈ 3.6) for drops¹ in fall under gravity. For drops in rise against gravity, the experimental data points here predict the value for the constant S as 0.3176 (≈ 0.32) $\text{m}^{-1} \text{s}^2$ which, for drops in fall under gravity has been predicted as 0.2483 (≈ 0.25) $\text{m}^{-1} \text{s}^2$ (ref. 1).

The experiments show that for drops (sufficiently small) in rise against gravity in the range of Reynolds number, R_e (from 25 to 495), Eotvos number, E_t [from (2×10^{-2}) to (70×10^{-2})] and Morton number, M_o [from (2.06×10^{-12}) to (7.13×10^{-10})] without exhibiting oscillation through an immiscible liquid column, the expressions suggested by Srinivasan and Satyanarayana¹ for drops in fall under gravity without exhibiting oscillation through an immiscible liquid column, hold good, viz.,

$$F = KD^2 u^2 \sigma (\eta / \sigma u D)^{1/2} (\rho / \sigma)^{1/2},$$

or (1)

$$F = KD^{3/2} u^{3/2} \eta^{1/2} \rho^{1/2}.$$

The method of arriving at these expressions has already been dealt with in detail¹. At the terminal velocity, for drops in rise against gravity without exhibiting oscillation through an immiscible liquid column, the buoyant force $(4\pi r^3 \sigma g / 3)$ of the liquid is equal to the sum of the weight of the drop, $(4\pi r^3 \rho g / 3)$ and the drag force F . That is,

$$4\pi r^3 \sigma g / 3 = (4\pi r^3 \rho g / 3) + F,$$

$$\therefore F = 4\pi r^3 (\sigma - \rho) g / 3. \quad (2)$$

Eq. (2) in eq. (1) gives

$$K = \sqrt{2} \pi g (r/u)^{3/2} (\sigma - \rho) / 3 \eta^{1/2} \rho^{1/2}, \quad (3)$$

$$u = (2\pi^2 g^2 r^3 (\sigma - \rho)^2 / 9 \eta K^2 \rho)^{1/3}, \quad (4)$$

$$\sqrt{2} \pi g S / 3 K = 1, \quad (5)$$

where

$$S = [(r/u)^{3/2} (\sigma - \rho)] / [\eta^{1/2} \rho^{1/2}], \quad (6)$$

and $g = 9.8 \text{ m s}^{-2}$, the acceleration due to gravity.

Eq. (6) (just as in the case of falling drop) is of vital importance in the sense that it may be used to determine the density ρ of the liquids which are lighter than the immiscible liquid column when conventional methods fail¹. Eq. (6) may be written as a quadratic equation from which

$$\rho = \{2\sigma + \lambda + [\lambda(\lambda + 4\sigma)]^{1/2}\} / 2, \quad (7)$$

where

$$\lambda = S^2 \eta / (r/u)^3. \quad (8)$$

S has been found to be approximately a constant for all systems studied here (see Table 3), the mean value of which is $0.3176 \text{ m}^{-1} \text{s}^2$.

Experimentally-observed data points predict a value for the constants S and K . The interval within which the predicted value of S and K holds good have to be ascertained for acceptability. Since an uncertainty analysis will help in establishing the uncertainty or error limits, which reveal the interval within which the predicted value of S and K holds good for acceptability, an attempt has been made here to adopt statistical method or technique²⁹⁻⁴⁰, which will enable to estimate and bring out the confidence limits (interval) within which the experimentally predicted value for S and K is an acceptable value of the parameters. Let $(x_1, \hat{y}_1), (x_2, \hat{y}_2), \dots (x_n, \hat{y}_n)$ be the n pairs of observations on the variables (observables) x and \hat{y} . Let the relationship between x and \hat{y} be of the form

$$\hat{Y} = \hat{A} + \hat{B}X. \quad (9)$$

Let the line of regression (best-fit)^{29,30} of \hat{Y} on X be

$$Y = A + BX. \quad (10)$$

Let the error estimate or residual^{29,30} as given by the line of best-fit (eq. (10)) be e , that is,

$$e = \hat{Y} - Y, \quad (11)$$

where Y is the estimated value as given by the line of best-fit (eq. (10)) for a given value of X . A (ref. 29) and B (ref. 29) are being estimated from

$$A = (\sum \hat{Y} - B \sum X) / n, \quad (12)$$

where

$$B = \{\sum X \hat{Y} - (\sum X \sum \hat{Y}) / n\} / \{\sum X^2 - (\sum X)^2 / n\}. \quad (13)$$

The expressions²⁹ giving $(1 - \alpha)$ per cent confidence limits for the regression parameters \hat{A} and \hat{B} are respectively,

$$\hat{A} = A \pm S_a t_{\alpha, n-2}, \quad (14)$$

$$\hat{B} = B \pm S_b t_{\alpha, n-2}, \quad (15)$$

where $t_{\alpha, n-2}$ is the table value for the two-tailed test at α level of significance and for $(n - 2)$ degrees of freedom. S_a and S_b are the standard error of A and B respectively, and they are expressed as²⁹

$$S_a = \{S_e^2 \{ (1/n) + [(\sum X/n)^2 / (\sum X^2 - ((\sum X)^2/n))] \}\}^{1/2}, \quad (16)$$

$$S_b = \{S_e^2 / \{\sum X^2 - ((\sum X)^2/n)\}\}^{1/2}. \quad (17)$$

S_e is the mean square error which is expressed as²⁹

$$S_e = \{ \{ [\sum \hat{Y}^2 - ((\sum \hat{Y})^2/n)] - B[\sum X \hat{Y} - ((\sum X \sum \hat{Y})/n)] \} / (n - 2) \}^{1/2}. \quad (18)$$

In the present study, eq. (6) may be written as

$$\rho^{1/2} / (\sigma - \rho) = 0 + (1/S) [(r/u)^{3/2} / \eta^{1/2}]. \quad (19)$$

This equation is of the form of eq. (9) and we get $\hat{Y} = \rho^{1/2} / (\sigma - \rho)$; and $X = (r/u)^{3/2} / \eta^{1/2}$. These in eqs 12, 13,

16, 17 and 18, give the estimated value of A , the estimated value of B $[=(1/S)_{\text{estimated}}]$, S_a , S_b and S_e respectively. Eqs (14) and (15) give $(1 - \alpha)$ percent confidence limits for regression parameters \hat{A} and \hat{B} respectively for $t_{\alpha, n-2}$ table value for the two tailed t -test at α level of significance of $(n - 2)$ degrees of freedom. It is obvious from eq. (15),

Highest confidence limit of

$$\hat{B} (= 1/S) = B + S_b t_{\alpha, n-2}, \quad (20)$$

Least confidence limit of

$$\hat{B} (= 1/S) = B - S_b t_{\alpha, n-2}, \quad (21)$$

Confidence interval

$$\hat{B} \text{ is } (B - S_b t_{\alpha, n-2}) \text{ to } (B + S_b t_{\alpha, n-2}), \quad (22)$$

Least confidence limit of

$$S = \{1/[B + S_b t_{\alpha, n-2}]\}, \quad (23)$$

Highest confidence limit of

$$S = \{1/[B - S_b t_{\alpha, n-2}]\}, \quad (24)$$

Confidence interval of S is

$$[1/(B + S_b t_{\alpha, n-2})] \text{ to } [1/(B - S_b t_{\alpha, n-2})]. \quad (25)$$

Since by eq. (5), $K = \sqrt{2\pi g S/3}$,

Least confidence limit of

$$K = (\sqrt{2\pi g/3})/[B + S_b t_{\alpha, n-2}], \quad (26)$$

Highest confidence limit of

$$K = (\sqrt{2\pi g/3})/[B - S_b t_{\alpha, n-2}], \quad (27)$$

Confidence interval of

$$K = (\sqrt{2\pi g/3})/[B + S_b t_{\alpha, n-2}] \text{ to } (\sqrt{2\pi g/3})/[B - S_b t_{\alpha, n-2}], \quad (28)$$

within these limits (interval) the experimentally predicted value for S and K is an acceptable value of the parameters S and K respectively.

A long graduated cylinder (diameter 5 cm) filled with the experimental liquid was used. Test liquid drops of known volume were gently injected at the bottom of the liquid column using a graduated Hamilton Precision micro-syringe. For injection, a small side tube attached to the graduated cylinder at the bottom and sealed with a septum was used. The rising drops and the liquids in the column were immiscible. Terminal velocity ' u ' was determined by observing the time ' t ' required by the liquid drop of radius ' r ' to cover the distance ' d ' between two graduations on the column. The drops studied here while rising are ellipsoidal in shape. If ' V ' is the volume of the drop of equivalent radius ' r ', then,

$$r = (3V/4\pi)^{1/3}.$$

The thirteen systems studied are given in Table 1. Density, viscosity and interfacial tension given in Table 1 were determined by the specific gravity bottle method, Ostwald viscometer and method of drops, respectively. As seen for drops in fall under gravity¹, here also, in the case of drops in rise against gravity, (r/u) is approximately constant (Table 2). Three data points each for seven liquid-drop pairs are alone given in Table 2. For the other liquid-drop pairs given in Table 1, the experimental results obtained show that the same (r/u) , approximately constant) holds good.

The value of S has been found to be approximately constant (Table 3) for all systems, just as observed for

Table 1. The systems and density, ratio of density, viscosity, interfacial tension and ratio of radius to terminal velocity values

Liquid drop	Column liquid	ρ (kgm ⁻³)	σ (kgm ⁻³)	σ/ρ (kgm ⁻³)	ρ/σ	Column liquid viscosity η (Nsm ⁻²)	Interfacial tension γ ($\times 10^{-3}$ Nm ⁻¹)	r/u (s)
Xylene	Water	857.95	1000.00	142.05	0.85795	0.001000	12.5	0.0160
Benzene	Water	870.78	1000.00	129.22	0.87078	0.001000	69.2	0.0174
Kerosene	Water	797.34	1000.00	202.66	0.79734	0.001000	43.8	0.0125
Turpentine	Water	860.03	1000.00	139.97	0.86003	0.001000	41.5	0.0165
Toluene	Water	860.89	1000.00	139.11	0.86089	0.001000	39.7	0.0165
Iso-amyl acetate	Water	882.15	1000.00	117.85	0.88215	0.001000	29.2	0.0185
Hexane	Water	665.37	1000.00	334.63	0.66537	0.001000	17.4	0.0085
Petroleum ether	Water	667.91	1000.00	332.09	0.66791	0.001000	24.3	0.0085
Cyclohexane	Water	775.04	1000.00	224.96	0.77504	0.001000	20.4	0.0116
Soap oil	Water	857.15	1000.00	142.85	0.85715	0.001000	34.2	0.0160
Heptane	Water	720.37	1000.00	279.63	0.72037	0.001000	38.7	0.0098
Water	Chlorobenzene	1000.00	1097.99	97.99	0.91076	0.000710	46.1	0.0196
Water	Bromobenzene	1000.00	1492.21	492.21	0.67015	0.000850	70.2	0.0071

r = radius of the liquid drop; u = terminal velocity of the drop; ρ = density of the liquid drop; σ = density of the liquid in the column; η = viscosity of the liquid in the column; γ = interfacial tension between the liquid drop and the liquid in the column.

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Table 2. Experimental data for liquid-drop pairs

Liquid - drop pair	Volume 'V' of the drop (μl)	Radius 'r' of the drop (× 10 ⁻⁴ m)	Distance 'd' travelled (× 10 ⁻² m)	Time 't' taken (s)	Observed terminal velocity 'u' (× 10 ⁻² ms ⁻¹)	r/u (s)	Reynolds number R _e	Eotvos number E _t (× 10 ⁻²)
Xylene in water Morton no. = 7.1275 × 10 ⁻¹⁰	0.5 1.0 2.0	4.9237 6.2035 7.8159	40 40 40	13.0 10.3 8.2	3.0769 3.8835 4.8780	0.0160 0.0160 0.0160	30.30 48.18 76.25	10.80 17.14 27.21
Benzene in water Morton no. = 3.8215 × 10 ⁻¹²	0.5 1.0 2.0	4.9237 6.2035 7.8159	40 40 40	14.1 11.2 8.9	2.8369 3.5714 4.4944	0.0174 0.0174 0.0174	27.94 44.31 70.26	1.77 2.82 4.47
Turpentine in water Morton no. = 1.9192 × 10 ⁻¹¹	0.5 1.0 2.0	4.9237 6.2035 7.8159	40 40 20	13.4 10.6 4.2	2.9851 3.7736 4.7619	0.0165 0.0164 0.0164	29.40 46.82 74.44	3.21 5.09 8.08
Iso-amyl acetate in water Morton no. = 4.6388 × 10 ⁻¹¹	0.5 1.0 2.0	4.9237 6.2035 7.8159	40 40 40	15.0 11.9 9.5	2.6667 3.3613 4.2105	0.0185 0.0185 0.0186	26.26 41.70 65.82	3.84 6.09 9.66
Hexane in water Morton no. = 6.2251 × 10 ⁻¹⁰	0.5 1.0 2.0	4.9237 6.2035 7.8159	40 40 40	6.9 5.5 4.4	5.7971 7.2727 9.0909	0.0085 0.0085 0.0086	57.09 90.23 142.11	18.28 29.01 46.05
Cyclohexane in water Morton no. = 2.5968 × 10 ⁻¹⁰	0.5 1.0 2.0	4.9237 6.2035 7.8159	40 40 40	9.4 7.5 5.9	4.2553 5.3333 6.7797	0.0116 0.0116 0.0115	41.90 66.17 105.98	10.48 16.64 26.41
Water in bromobenzene Morton no. = 3.2687 × 10 ⁻¹²	0.5 1.0 2.0	4.9237 6.2035 7.8159	40 40 40	5.8 4.6 3.7	6.8966 8.6957 10.8108	0.0071 0.0071 0.0072	119.22 189.40 296.67	6.66 10.58 16.79

Table 3. The value of $(r/u)^{3/2}/\eta^{1/2}$ and $\rho^{1/2}/(\sigma-\rho)$ and the constant K obtained by using eq. (3)

Liquid drop	Column liquid	$X = (r/u)^{3/2}/\eta^{1/2}$	$\hat{Y} = \rho^{1/2}/(\sigma-\rho)$	S^+	K*	Y**	e***
Xylene	Water	0.0640	0.2062	0.3104	4.5046	0.2019	0.00425
Benzene	Water	0.0726	0.2284	0.3178	4.6128	0.2291	-0.00070
Kerosene	Water	0.0442	0.1393	0.3172	4.6034	0.1394	-0.00003
Turpentine	Water	0.0670	0.2095	0.3199	4.6427	0.2115	-0.00198
Toluene	Water	0.0670	0.2109	0.3178	4.6119	0.2115	-0.00058
Iso-amyl acetate	Water	0.0796	0.2520	0.3157	4.5823	0.2512	0.00087
Hexane	Water	0.0248	0.0771	0.3215	4.6659	0.0780	-0.00093
Petroleum ether	Water	0.0248	0.0778	0.3184	4.6216	0.0780	-0.00019
Cyclohexane	Water	0.0395	0.1238	0.3192	4.6334	0.1245	-0.00080
Soap oil	Water	0.0640	0.2050	0.3123	4.5321	0.2019	0.00300
Heptane	Water	0.0307	0.0960	0.3196	4.6389	0.0966	-0.00067
Water	Chlorobenzene	0.1030	0.3227	0.3191	4.6313	0.3251	-0.00241
Water	Bromobenzene	0.0205	0.0642	0.3194	4.6355	0.0645	-0.00030
	Mean			0.3176	4.6090		

*The constant of eq. (3); *S of eq. (6); **By eq. (10) $Y = A + BX$

By eq. (12) using data points given in Table 4, $A = -0.0003$

By eq. (13) using data points given in Table 4, $B = 3.1601$

***By eq. (11) $e = \hat{Y} - Y$

From Figure 1, $S = 0.3175 \text{ m}^{-1} \text{ s}^2$; $K = 4.6074$

Estimated value of $K = \sqrt{2\pi g/3B} = 4.5920$; $g = 9.8 \text{ m s}^{-2}$; (r/u) , ρ , σ , and η from Table 1.

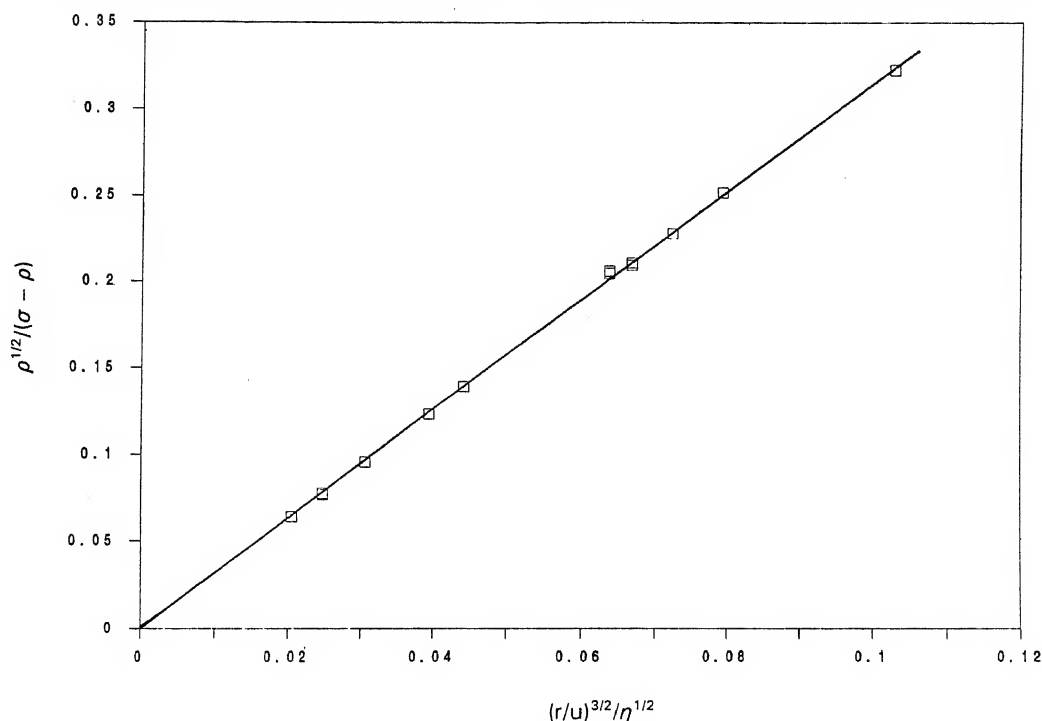


Figure 1. Plot of data given in Table 3.

drops in fall under gravity¹. Experimental data points given in the fourth and fifth columns of Table 3 in eq. (6) give S of which $S_{\text{mean}} = 0.3176 \text{ m}^{-1} \text{ s}^2$ (Table 3). Comparison with drops in fall under gravity¹ (where the mean value of S is $0.2483 \text{ m}^{-1} \text{ s}^2$) clearly shows that the value of S remains positive for drops in rise against gravity as observed for drops in fall under gravity¹. The expression revealing the relation between S and K , here eq. (5), is the same as eq. (7) given by Srinivasan and Satyanarayana¹ and also for expressions ρ and λ .

Comparison of eqs (2)–(4), (6) and with those of expressions, F , K , u and S given by Srinivasan and Satyanarayana¹, shows that they are of similar form except for the fact that ρ and σ have just interchanged their positions.

S (eq. (6)) involves two terms $(r/u)^{3/2}/\eta^{1/2}$ and $\rho^{1/2}/(\rho - \sigma)$. The values of these terms are given in Table 3. A graph (Figure 1) is drawn with $(r/u)^{3/2}/\eta^{1/2}$ along the X-axis and $\rho^{1/2}/(\sigma - \rho)$ along the Y-axis. Referring to eq. (3), it may be seen that the slope of the line in the graph is $\sqrt{2\pi g/3K}$ which works out to be 3.1500. The slope value leads to $K = 4.6074$. This slope value, by eq. (5), i.e., $\sqrt{2\pi g S/3K} = 1$ gives $S = 0.3175 \text{ m}^{-1} \text{ s}^2$.

Experimental values of $(r/u)^{3/2}/\eta^{1/2}$ and $\rho^{1/2}/(\sigma - \rho)$ given in Table 3, in eq. (3), give the value of K of which $K_{\text{mean}} = 4.6090$ (Table 3). Eq. (5) may also be used to determine K and K_{mean} .

While attempting to predict values for S and K , it becomes necessary to carry out uncertainty analysis which will help in establishing with experimental data points, the uncertainty or error limits of S and K . Therefore, as a point of interest, error analysis has also been carried

out here, with experimental data points to estimate confidence limits (interval) of S and K , and to find out the error estimate 'e'.

With $n = 13$ and using the data given in Table 3, viz. $X = (r/u)^{3/2}/\eta^{1/2}$ and $\hat{Y} = \rho^{1/2}/(\sigma - \rho)$, or data points given in Table 4 in eqs (12), (13), (16), (17) and (18) give respectively

$$A = -0.0003,$$

$$B = 3.1601,$$

$$S_a = 0.0013,$$

$$S_b = 0.0220,$$

$$S_e = 0.0019.$$

$1/B$ gives the estimated value of S which is 0.3164. This value is nearly equal to the observed value of S given in the 6th column of Table 3 with negligible percentage of error. Likewise, $\sqrt{2\pi g/3B}$ gives the estimated value of K which is 4.5920. This value differs with negligible percentage of error with the observed values of K given in the 7th column of Table 3. The error estimate or residual 'e' is given in Table 3. From Table 3, it may be seen that 'e' is negligibly small.

Now, for $t_{\alpha, n-2}$, the table value of t for $n - 2 (= 11)$ degrees of freedom and α (5, 2, 1 and 0.1) per cent level of significance²⁹ $t_{\alpha, 11}$ are respectively 2.201, 2.718, 3.106 and 4.437 for the two-tailed t -test. For these values, with value $B = 3.1601$ and $S_b = 0.0220$, eqs 20–28 give the significant values related to the confidence limits (interval) of \hat{B} , S and K are as shown in Table 5.

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Table 3 gives mean observed value of $S = 0.3176 \text{ m}^{-1} \text{ s}^2$; $K = 4.6090$. Figure 1 gives $S = 0.3175 \text{ m}^{-1} \text{ s}^2$ and $K = 4.6074$. The error analysis giving significant values regarding confidence limits of S and K are given in Table 5. The values of S and K agree quite reasonably.

The estimated value, $0.3164 \text{ m}^{-1} \text{ s}^2$, the significant values of the enumerated confidence limits (interval) of the parameter S (Table 5) indicate that (i) $S = 0.3176 \text{ m}^{-1} \text{ s}^2$ (Table 3, the mean observed value experimentally predicted for the parameter S) is acceptable and (ii) by virtue of the importance of eq. (6), (the value of the parameter $S = 0.3176 \text{ m}^{-1} \text{ s}^2$) it is possible

to get fairly accurate density values by eqs (7) and (8) for liquids which are lighter than the immiscible liquids in the column and available in small quantities just as observed in the case of falling drops¹.

The values of K determined from the graph (Figure 1) and that determined from eq. (3) (Table 3) are 4.6074 and 4.6090 respectively and the mean of both (observed) is 4.6082. That is, 4.6082 is the value experimentally predicted for the parameter K . Looking at the estimated value, 4.5920, the significant values of the enumerated confidence limits (interval) of the parameter K (Table 5), it appears that (i) the value 4.6082, experi-

Table 4. Data prepared for error analysis

Liquid drop	$X = (r/u)^{3/2}/\eta^{1/2}$	$\hat{Y} = \rho^{1/2}/(\sigma - \rho)$	X^2	\hat{Y}^2	$X\hat{Y}$
Xylene	0.0640	0.2062	0.0041	0.0425	0.0132
Benzene	0.0726	0.2284	0.0053	0.0521	0.0166
Kerosene	0.0442	0.1393	0.0020	0.0194	0.0062
Turpentine	0.0670	0.2095	0.0045	0.0439	0.0140
Toluene	0.0670	0.2109	0.0045	0.0445	0.0141
Iso-amyl acetate	0.0796	0.2520	0.0063	0.0635	0.0201
Hexane	0.0248	0.0771	0.0006	0.0059	0.0019
Petroleum ether	0.0248	0.0778	0.0006	0.0061	0.0019
Cyclohexane	0.0395	0.1238	0.0016	0.0153	0.0049
Soap oil	0.0640	0.2050	0.0041	0.0420	0.0131
Heptane	0.0307	0.0960	0.0009	0.0092	0.0029
Water	0.1030	0.3227	0.0106	0.1041	0.0332
Water	0.0205	0.0642	0.0004	0.0041	0.0013
	$\Sigma X = 0.7016$	$\Sigma \hat{Y} = 2.2129$	$\Sigma X^2 = 0.0455$	$\Sigma \hat{Y}^2 = 0.4528$	$\Sigma X\hat{Y} = 0.1435$

$X = (r/u)^{3/2}/\eta^{1/2}$ from Table 3; $\hat{Y} = \rho^{1/2}/(\sigma - \rho)$ from Table 3.

Table 5. Error analysis - Enumerated significant values (confidence limits - interval) of \hat{B} , S and K

$t_{\alpha, n-2}$	Confidence limits of \hat{B}		Confidence interval of \hat{B}	Confidence limits of S		Confidence interval of S	Confidence limits of K		Confidence interval of K
	Least (eq. (21))	Highest (eq. (20))		Least (eq. (23))	Highest (eq. (24))		Least (eq. (26))	Highest (eq. (27))	
$t_{0.05, 11}$	3.1117	3.2085	0.0965	0.3117	0.3213	0.0096	4.5238	4.6632	0.1394
2.201*	(3.11)	(3.21)	(0.097)	(0.312)	(0.321)	(0.010)	(4.52)	(4.66)	(0.139)
$t_{0.02, 11}$	3.1003	3.2199	0.1196	0.3106	0.3225	0.0119	4.5079	4.6806	0.1727
2.718*	(3.10)	(3.22)	(0.120)	(0.311)	(0.323)	(0.012)	(4.51)	(4.68)	(0.173)
$t_{0.01, 11}$	3.0918	3.2284	0.1366	0.3097	0.3234	0.0137	4.4948	4.6942	0.1994
3.106*	(3.09)	(3.22)	(0.137)	(0.310)	(0.323)	(0.014)	(4.49)	(4.69)	(0.199)
$t_{0.001, 11}$	3.0625	3.2577	0.1952	0.3070	0.3265	0.0195	4.4551	4.7391	0.2840
4.437*	(3.06)	(3.26)	(0.195)	(0.307)	(0.327)	(0.020)	(4.46)	(4.74)	(0.284)

*Significant value of $t_{\alpha, n-2}$ from Table (ref. 29).

Estimated value of $B = 3.1601$ (By eq. (13) using data points given in Table 4); Estimated value of $S = 1/B = 0.3164$; Estimated value of $K = \sqrt{2\pi g/3B} = 4.5920$; $g = 9.8 \text{ m s}^{-2}$

Using data points given in Table 4, by eq. (16), $S_a = -0.0013$ (= 0.001); By eq. (17), $S_b = 0.02220$ (0.022); by eq. (18), $S_c = 0.0019$ (= 0.002)

At $t_{0.05, 11}$, $\hat{B} = 3.1601 \pm 0.0484$, $S = 0.3165 \pm 0.0048$, $K = 4.5935 \pm 0.0697$
(3.16 \pm 0.048) (0.317 \pm 0.005) (4.59 \pm 0.070)

At $t_{0.02, 11}$, $\hat{B} = 3.1601 \pm 0.0598$, $S = 0.3166 \pm 0.0060$, $K = 4.5943 \pm 0.0864$
(3.16 \pm 0.060) (0.317 \pm 0.006) (4.59 \pm 0.086)

At $t_{0.01, 11}$, $\hat{B} = 3.1606 \pm 0.0688$, $S = 0.3165 \pm 0.0068$, $K = 4.5945 \pm 0.0999$
(3.16 \pm 0.069) (0.317 \pm 0.007) (4.60 \pm 0.100)

At $t_{0.001, 11}$, $\hat{B} = 3.1601 \pm 0.0976$, $S = 0.3168 \pm 0.0098$, $K = 4.5971 \pm 0.1420$
(3.16 \pm 0.098) (0.317 \pm 0.010) (4.60 \pm 0.142)

The mean observed value experimentally predicted (i) for $S = 0.3176$ (= 0.318) $\text{m}^{-1} \text{ s}^2$ (Table 3, Mean value 0.3176 $\text{m}^{-1} \text{ s}^2$; From Figure 1, 0.3175 $\text{m}^{-1} \text{ s}^2$); (ii) for $K = 4.6082$ (= 4.61) (Table 3, Mean value 4.6090; From Figure 1, 4.6074).

In the brackets, the values are given in three significant figures for easy perusal of the error limits.

mentally predicted for the parameter K is acceptable (ii) and hence, for the motion of a liquid drop in rise or rising upward against gravity through an immiscible liquid column without exhibiting oscillation of fairly high Reynolds number, Re (from 25 to 495), Eotvos number, E_t [from (2×10^{-2}) to (70×10^{-2})] and Morton number, Mo [from (2.06×10^{-12}) to (7.13×10^{-10})], the experimentally predicted value 4.6082 for the constant K in the drag force expression eq. (1) is acceptable.

If only three significant figures (given in brackets in Table 5) for the parameters S and K are considered, it may be seen that the error limits lie on either side of the value of $S = 0.317 \text{ m}^{-1} \text{ s}^2$ and for the value of $K = 4.59$ or 4.60, the mean of which is 4.60. The experimentally observed value for S and K when expressed up to three significant figures, become $0.318 \text{ m}^{-1} \text{ s}^2$ and 4.61 respectively. That is, they predict in two significant figures $0.32 \text{ m}^{-1} \text{ s}^2$ for S and 4.6 for K . In other words, in the drag force expression eq. (1), the value of K is 4.6.

The motion of the drop in rise against gravity may be thought to be just opposite to that of the motion of a drop in fall under gravity. The results for the drops considered in rise against gravity show that the proportionality constant K in the drag force expression (eq. (1)) is 4.6082 and the mean value of the constant S (eq. (6)) is $0.3176 \text{ m}^{-1} \text{ s}^2$. In the case of falling drop, for drops in fall under gravity¹, the value of K and S are respectively 3.6094 and $0.2483 \text{ m}^{-1} \text{ s}^2$. A comparison shows that values obtained for K and S for drops in rise against gravity differ from those values obtained for drop in fall under gravity¹ and furthermore, K and S remain positive for both viz., drop in rise against gravity and drop in fall under gravity¹.

Eq. (4) with $K = 4.6082$ may be used to predict the terminal velocity (just as seen for drop in fall under gravity¹) of the drop rising against gravity without exhibiting oscillation if r , η , σ and ρ are known. Using eqs (7) and (8) with the mean experimental value of $S = 0.3176 \text{ m}^{-1} \text{ s}^2$ (Table 3), if r , u , η and σ are known, one may determine (as seen for drop in fall under gravity¹) density ρ of the drops for which density cannot be determined by the capillary tube method where weighing is a problem or by any other conventional method¹.

Error analysis (confidence limits/interval) indicates that (i) getting fairly accurate value for density ρ with $S = 0.3176 \text{ m}^{-1} \text{ s}^2$ is possible and (ii) for the fresh drag force expression eq. (1), the experimentally predicted value 4.6082 for the constant K is acceptable.

If only two significant figures are considered, the observed value, the estimated value and the central value about which error limits lie, show that $S = 0.32 \text{ m}^{-1} \text{ s}^2$ and $K = 4.6$.

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Inter-conversion of chemiosmotic parameters and its inhibition in *Thiobacillus ferrooxidans*

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In *Thiobacillus ferrooxidans*, an iron oxidizing obligatory acidophilic bacterium, bioenergetics was found to be critically dependent on sulphate distribution across its membrane. Under physiological condition, the sulphate distribution was found to be far from its electrochemical equilibrium, and a high residual proton motive force (~110 mV) remained conserved under varying external pH even in respiration-starved cells. The inter-conversion between bioenergetic parameters, namely membrane potential and pH gradient, was inhibited at a critical, low pH. Below this critical pH, proton-coupled sulphate influx was observed, as measured by radio-labelled transport assay, leading to collapse of the two chemiosmotic gradients. The associated alteration of membrane structure and permeability property was monitored by intrinsic fluorescence of *T. ferrooxidans* and extrinsic fluorescent probes.

ACIDOPHILIC bacteria have the remarkable ability to maintain their cytoplasmic pH close to neutrality even when external pH is below 4 (refs 1, 2). These cells, thus, can be taken as model system of chemiosmotic theory *in extremis*. According to this theory, the primary free energy available to a cell is its proton motive force (PMF)³, which is composed of two parameters, namely the chemical gradient and the electrical gradient of proton:

$$\Delta p = 60 \Delta \text{pH} - \Delta \psi, \quad (1)$$

where $\Delta \psi$ and ΔpH are membrane potential and pH gradient across the energy-transducing membrane, respectively. Obviously, chemiosmotic theory demands a mechanism of inter-conversion between these two parameters so that PMF can be kept constant. Moreover, since cytoplasmic pH is fairly conserved, the membrane potential varies under different physiological conditions. Most of the acidophiles, including *T. ferrooxidans*, are reported to have inside positive $\Delta \psi$; generation of membrane potential is observed in the presence of protonophores or in respiration-inhibited cells⁴⁻⁸. The respiratory chain of *T. ferrooxidans* utilizes oxidation of Fe^{2+} to Fe^{3+} , and therefore, the available free energy is so small that cytochrome oxidase cannot pump protons from the cytoplasm to the external medium^{9,10}. Thus, an efficient mechanism for respiration-independent ΔpH -

$\Delta \psi$ inter-conversion may be present in this organism, which is yet to be reported explicitly.

The initial objective of this work was to detect physiological limits of the interconvertibility of the two bioenergetic parameters. Our preliminary observation indicates that there exists a critical external pH, at which the inter-conversion fails¹¹. The mechanism of such failure may provide important insights towards better understanding of bioenergetic regulation in *T. ferrooxidans*.

T. ferrooxidans (ATCC 23270) was grown in 9 K medium¹² at pH 2.0 with aeration. Cells were harvested by centrifugation at 10,000 g for 15 min at 25°C, washed in ferrous free 9 K medium (9 K⁻) and 10 mM sulphuric acid (pH 2.0), filtered through a Whatman No. 1 paper and remaining trace of iron precipitate was removed by centrifugation on silicone oil (high density, $\rho = 1.050$) at 1,000 g for 3 min.

Membrane potential and internal pH were determined by using S^{14}CN^- and $[\text{C}^{14}]\text{acetate}$ by rapid centrifugation method^{4,13}. For measurement of membrane potential, cells at protein concentration of about 5 mg/ml were incubated with $^3\text{H}_2\text{O}$ (5 $\mu\text{Ci/ml}$) and KS^{14}CN (0.2 $\mu\text{Ci/ml}$). For pH gradient, $\text{Na}-[\text{C}^{14}]\text{acetate}$ (0.2 $\mu\text{Ci/ml}$) or $[\text{C}^{14}]\text{chloroacetate}$ (0.2 $\mu\text{Ci/ml}$) were used. After dual incubation with $^3\text{H}_2\text{O}$ and the probe for membrane potential or pH gradient for 5 min at room temperature, the cells were separated by rapid centrifugation through dibutyl phthalate ($\rho = 1.043$) at 12,000 g for 5 min. The bacterial pellet was dissolved in 1% SDS, diluted in scintillation cocktail-W and counts were taken in a liquid scintillation counter (Model LS 5000 TD, Beckman). The membrane potential and pH gradient were calculated using the following equations:

$$\Delta \psi = 60 \log \{ [\text{SCN}]_{\text{in}} / [\text{SCN}]_{\text{out}} \}, \quad (2)$$

$$\Delta \text{pH} = \log \{ [\text{A}]_{\text{in}} / [\text{A}]_{\text{out}} (1 + 10^{\text{pH}_{\text{out}} - \text{pK}_a}) - 1 \} + \text{pK}_a - \text{pH}_{\text{out}}, \quad (3)$$

where the subscripts 'in' and 'out' represent concentrations in the cytoplasm and the supernatant, respectively.

To measure internal buffering capacity of the whole cells and proton flux across their membrane, a procedure described previously⁸ was followed. Cells permeabilized with 10% Triton X-100 were suspended in water at concentration 3.5 mg/ml. After each addition of acid pulse of 2.5–10 μl 20 m(N) HCl, pH was measured by an expandable ion analyser (Model EA920, Orion Research Inc., USA). 10% Triton X-100 and intact cells suspended in water were used as two controls. The value of the buffering capacity was expressed in nmole of proton required to change the pH by one unit per mg of cell protein. To measure proton flux across membrane, cells at 3.5 mg/ml were suspended in unbuffered assay medium (dilute H_2SO_4) and treated as required. pH was

monitored at intervals and proton influx was calculated from alkalization of the external medium⁸.

Cells were suspended in 10 mM Tris-Cl (pH 7.0) containing 0.4 M sucrose and 20 mM EDTA and then treated with lysozyme at 0.2 mg/ml for 30 min. The reaction mixture was diluted with 0.4 M sucrose containing 10 mM MgCl₂. Swelling of the pre-formed spheroplasts was monitored by absorbance at 600 nm or by using ³H₂O.

The fluorescence experiments were carried out in a Shimadzu RF-540 spectrofluorometer at room temperature. Whole cells at 50 µg/ml were excited at 295 nm (2.5 nm slit) and the fluorescence spectra were recorded in the wavelength range 300–380 nm (2.5 nm slit). Quenching experiments were performed with excitation at 295 nm (slit 2 nm) and emission at 335 nm (slit 2–5) with increasing concentrations of quenchers: acrylamide 33.3–200 mM, copper sulphate 5–30 mM and potassium iodide 5–30 mM. Stern–Volmer constants were calculated from the slopes of F_0/F_1 vs $[Q]$ plots, where F_0 and F_1 were the unquenched and the quenched fluorescence intensities, respectively, and $[Q]$ was the quencher concentration¹⁴.

The cells at 100 µg/ml were incubated with 10 µM of 8-anilino-1-naphthalene sulphonate (ANS) for 15 min and the fluorescence spectra were obtained with maximum intensity of excitation at 365 nm (2.5 nm slit) and emission wavelength range 400–550 nm (2.5 nm slit).

The cells without or with protonophore were incubated with [³⁵S]sulphuric acid. Then they were isolated by oil centrifugation and isotope distribution in the pellet and the supernatant was measured at different time intervals. To calculate internal concentration of sulphate, the internal volume was determined by ³H₂O distribution.

The earlier reports on bioenergetics of *Thiobacilli* have claimed that maintenance of internal pH in the acidophiles is not an energy-dependent process^{4,8}. In respiration-inhibited *T. ferrooxidans* and *T. acidophilus*, PMF of about 58 mV and 36 mV have been reported, while in the active cells the pmf values were 256 mV and 90 mV, respectively^{4,5}. In both the cases cytoplasmic acidification was only by 0.4 pH unit. We report here existence of a protonophore-sensitive PMF and its conservation over a pH range in the non-respiring *T. ferrooxidans* cells. When *T. ferrooxidans* cells were incubated in 9K⁺ medium of pH 1.8 to pH 3.4, membrane potential and pH gradient were found to decrease with increasing external pH (Figure 1), with pmf remaining conserved at about 110 mV. When the external medium was replaced by β -alanine sulphate buffer of respective pH, the values of these parameters remained virtually the same. Over the pH range under study, therefore, the $\Delta\psi$ – Δ pH inter-conversion is neither a respiration-dependent process nor dependent on alkali-uptake by the cells.

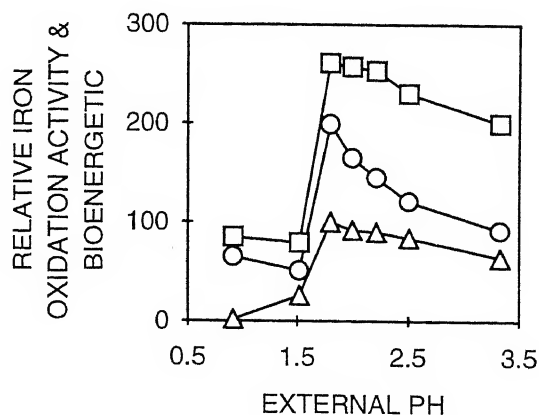


Figure 1. pH profiles of relative ferrous oxidation activity and bioenergetic parameters of *T. ferrooxidans*. Oxidation activity (Δ), membrane potential (O) and pH gradient (□) were measured in non-respiring condition as described in text. The cells were starved for about 6 h during their harvesting and purification.

As pointed out by several workers, the redox span (ΔE_h) between $\text{Fe}^{2+}/\text{Fe}^{3+}$ ($E_{m,2} = 780$ mV) and $\text{H}^+/\text{H}_2\text{O}$ ($E_{m,6.5} = 850$ mV) couples is only 70 mV and so in the respiring cells (where the membrane potential is small and PMF is high⁴), the electron transfer provides little scope for proton pump activity. However, this report indicates that the bioenergetics of the non-respiring cells might be qualitatively different. The large membrane potential that builds up in non-respiring cells is to be added to the redox span¹⁵, giving a Gibbs free energy change per electron translocation:

$$\Delta G = -F(\Delta E_h + \Delta\psi), \quad (4)$$

where F is the Faraday constant. Thus, respiration at pH 2.0 is expected to yield 22.7 kJ mol^{-1} in these cells compared to only 6.8 kJ mol^{-1} in the respiring cells. Though the implication of this is not yet clear, the generation of inside-positive membrane potential upon respiration deprivation appears to be thermodynamically beneficial, though perhaps transiently.

At very low pH (below pH 1.8), however, $\Delta\psi$ decreased discontinuously to a baseline at 65–70 mV. pH shift experiments indicated that after a brief exposure to such low pH, cells failed to regain high $\Delta\psi$ or to oxidize ferrous even when shifted to pH 2.0.

T. ferrooxidans showed typical pH dependence in its iron-oxidizing activity with a maximum near pH 2.0, its physiological pH (Figure 1). A comparison of the pH profile of iron oxidation and that of $\Delta\psi$ reveals remarkable similarity.

At pH 3.4 after treatment with 200 µM of 2,4-dinitrophenol (DNP), $\Delta\psi$ increased from 93 mV to 142 mV. These DNP-treated cells could respire as evident from ferrous oxidation after they were re-suspended at

pH 2.0. The value of cytoplasmic buffering capacity was found to be 95 nmole of proton per pH unit per mg of protein, while DNP-mediated proton influx was 65 nmole per mg of cell protein. Therefore, the cytoplasm should have been acidified by about 0.7 pH unit, which was exactly what was found by radio-isotopic measurement of pH gradient. Thus, though protonophore treatment gave rise to almost complete abolition of pmf, an appreciable Δ pH remained. The protonophore appears to introduce a proton influx that attains electrochemical equilibrium and becomes self-limiting in the absence of compensatory charge translocation^{2,8}. Under this condition, internal buffering capacity could provide protection against internal acidification. This passive mechanism of pH homeostasis is therefore important even in the non-respiring cells where increasing amount of proton remains unabated in the cytoplasm due to non-functioning terminal oxidase. Since the proton influx was comparable with the amount of DNP in the assay medium (57 nmole/mg of cell protein), prevention of reverse flow of the 'uncoupler' by inside-positive membrane potential suggested earlier¹⁶ seems to be operative.

Effect of protonophore treatment was, however, found to be external-pH dependent. At pH 1.8, for example, treatment with 200 μ M of DNP resulted in abolition of pH gradient. The cells were unable to respire after DNP was washed away. Interestingly, the change in the other chemiosmotic parameter was anomalous – $\Delta\psi$ was found to decrease from 199 mV to 68 mV. Collapse of $\Delta\psi$ in presence of protonophore has been reported in at least another acidophile and interpreted as non-specific disruption of membrane at high DNP concentration⁷.

Protonophore-induced collapse of the membrane potential, appreciable proton influx and inactivation of cells near pH 2 could only be accounted for by considering translocation of charges other than proton. Presumably, anion of assay medium could provide a compensatory conductance to proton influx under this condition.

Upon excitation at 275 nm or 295 nm, *T. ferrooxidans* cells showed an intrinsic fluorescence spectra with emission maximum at about 338 nm, presumably blue-shifted spectra of tryptophan (Trp) residues¹⁴. Comparison of relative intensity of intrinsic fluorescence of the membrane, its high-ionic strength removable and non-removable fractions, and cytoplasmic fraction showed the whole cell fluorescence was predominantly due to integral membrane protein¹¹. When external pH was varied from 1.1 to 3.5, the fluorescence intensity was found to increase with increasing pH with no appreciable shift in wavelength maximum (Figure 2a). Invariant emission maximum over the pH range indicates that the Trp residues were in a similar environment. Ionic collision quenchers of membrane-bound fluorophores have been used to monitor their accessibility to the fluoro-

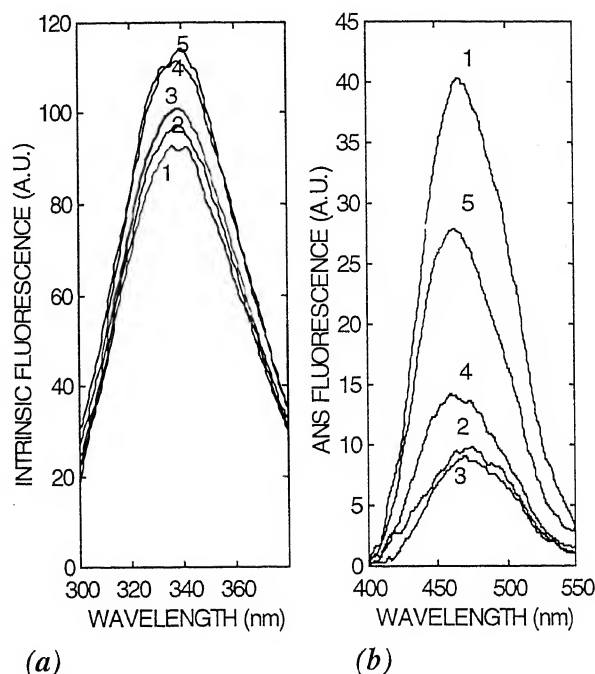


Figure 2. Emission spectra of intrinsic fluorescence (a) and ANS fluorescence (b). External pH values were pH 1.1 (1), pH 1.5 (2), pH 1.9 (3), pH 2.9 (4) and pH 3.5 (5). Each intrinsic fluorescence spectrum was corrected for background fluorescence of buffer, and each ANS spectrum (ANS concentration 10 μ M) was corrected for buffer, free ANS and cells.

phore site^{14,17}. Stern–Volmer plots for various quenchers of Trp were found to be linear, suggesting one class of fluorophores (Figure 3). Over the pH range of 1.1 to 3.5, the Stern–Volmer constants (K_{SV}) for acrylamide and Cu^{++} were $3.04 \pm 0.18 \text{ M}^{-1}$ and $9.17 \pm 1.27 \text{ M}^{-1}$, respectively (Figure 3a and b). For I^- , however, K_{SV} increased with decreasing pH, dramatically near pH 1.6 (Figure 3c). Though K_{SV} of acrylamide and copper varied in narrow ranges, increase in the iodide-quenching constant at low pH suggests that the intrinsic fluorophores in the whole cells became more accessible to added anions.

Binding studies of ANS have been used to determine fixed charges on the membrane surface¹⁸. The pH profile of ANS fluorescence intensity showed discontinuity between pH 1.5 and 1.9 (Figure 2b). Below pH 1.4, slight red-shift in the emission spectra was also observed. The cells pre-incubated in such a low pH and then shifted to higher pH (i.e. pH 2.0) showed ANS spectrum similar to those suspended at the former pH. Thus, ANS fluorescence appears to monitor some irreversible alteration in the membrane structure at low pH.

Preliminary permeability study with spheroplasts showed osmotic swelling at higher concentrations of sulphate (Table 1), suggesting influx of sulphate and its counter-ion. The direct assay revealed a fast followed

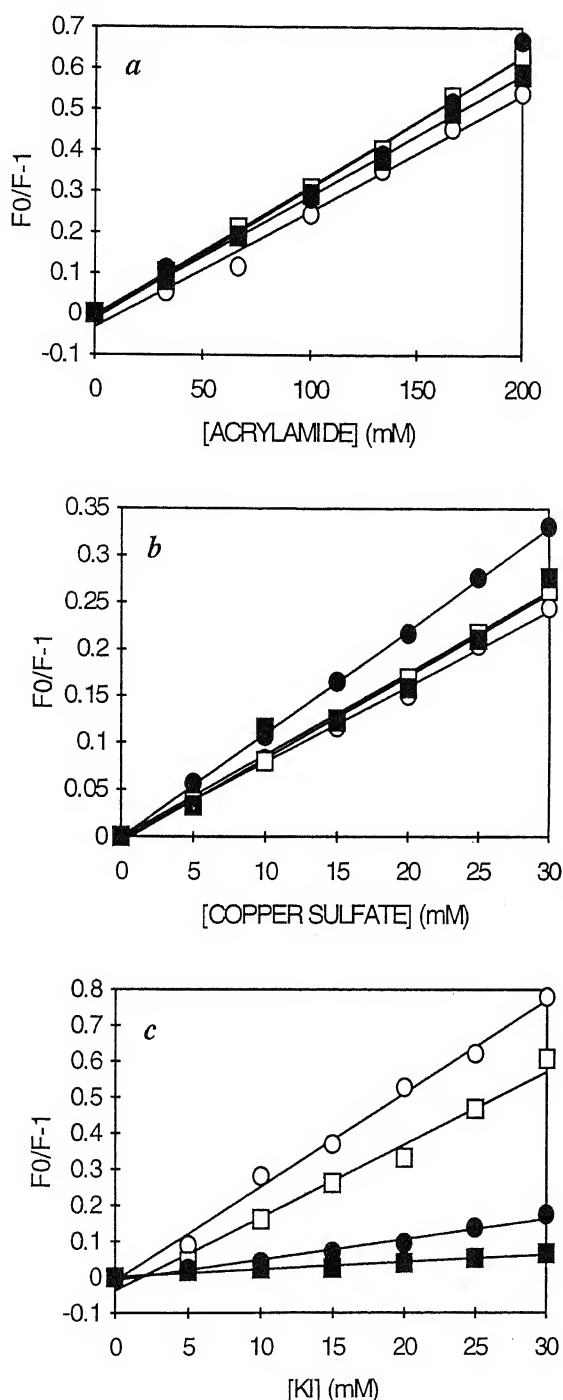


Figure 3. Stern-Volmer plots for quenching of intrinsic fluorescence with acrylamide (a), copper (b) and iodide (c). pH 1.4: (O); pH 1.6 (□); pH 1.9: (●); pH 3.3: (■). Linear correlation coefficients were greater than 0.995.

by a slow sulphate distribution in the cells (Figure 4). At pH 2.0, the sulphate concentration in DNP-treated cells was about twice as much as that in the control. This is in conformation with our observation regarding anomalous changes in the bioenergetic parameters and compensatory anionic charge movement. Assuming the

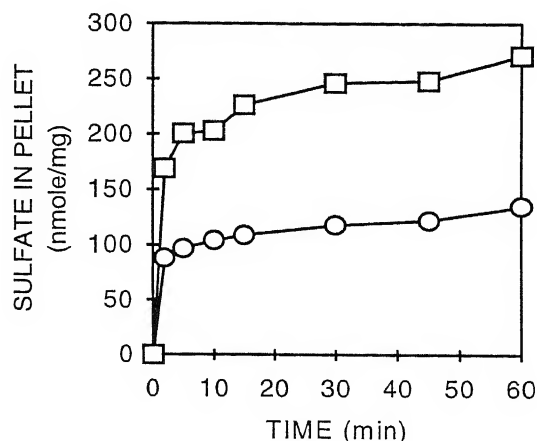


Figure 4. Time kinetics of sulphate accumulation. Cells suspended in $9K^+$ medium (pH 2.0) at protein concentration of 5 mg/ml were incubated without or with 200 μ M DNP for 15 min and [^{35}S]sulphuric acid at final concentration of 0.05 μ Ci/ml was added to each tube at $t=0$ min. Pellet counts of aliquots were taken at intervals. Control (O), DNP treated (□).

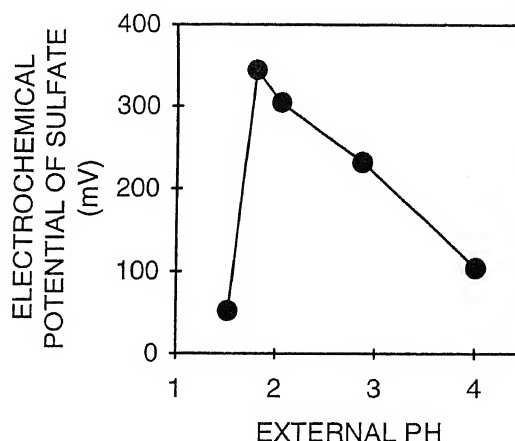


Figure 5. pH profile of the electrochemical potential of sulphate ion across the membrane. The parameter was measured from sulphate distribution ratio and membrane potential: $\Delta\mu_{SO_4} = 2\Delta\psi - 60 \log ([SO_4]_{in}/[SO_4]_{out})$.

Table 1. Effect of sulphate on spheroplast swelling. For optical experiments, spheroplast preparation was suspended at protein concentration of 50 μ g/ml and incubated for 5 min with increasing sulphate concentration. For direct measurement of volume, the spheroplasts were suspended at protein concentration of 7 mg/ml in various sulphate concentrations for 5 min and the volume was obtained with 3H_2O

Sulphuric acid concentration (mM)	Absorbance at 600 nm	Volume (μ l/mg of protein)
10	0.185 ± 0.01	3.05 ± 0.09
100	0.182 ± 0.02	3.21 ± 0.04
200	0.172 ± 0.02	3.80 ± 0.10

\pm indicates the standard deviations from mean values.

Nernst equilibrium distribution of SO_4^{2-} , the expected distribution ratios ($[SO_4]_{in}/[SO_4]_{out}$) are 3.2×10^5 and 54

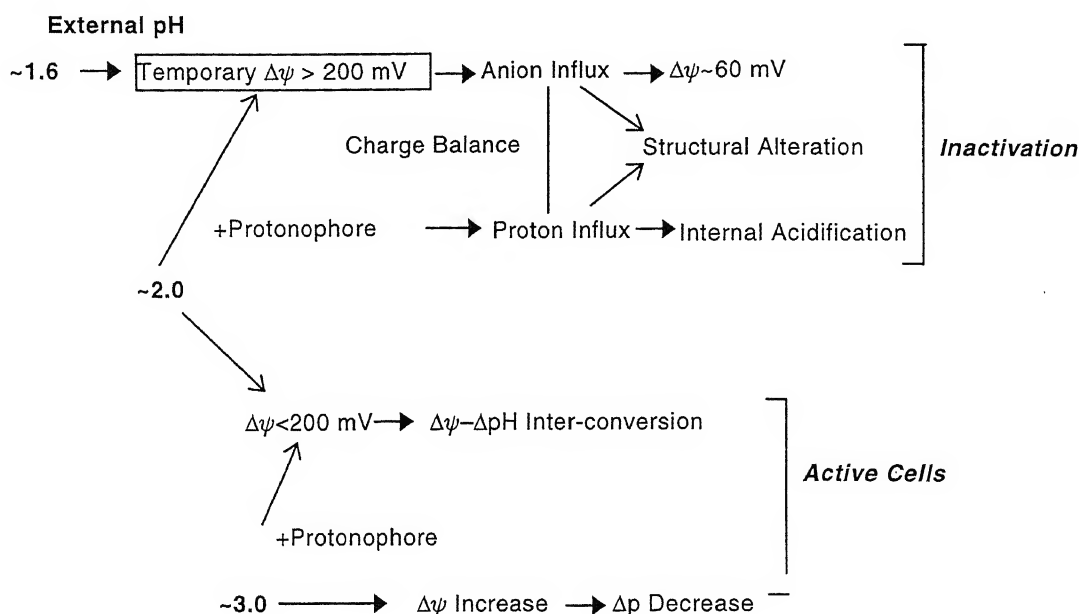


Figure 6. Scheme for relationship between bioenergetic parameters and ion uptake in *T. ferrooxidans*. Existence of a critical, high membrane potential has been proposed, above which the membrane breakdown phenomenon was observed.

at membrane potential of 165 and 52 mV, respectively. Thus, the sulphate ions are subjected to a high $\Delta\psi$ driven inward drag in the active cells, but not in the inactive cells.

The pH dependence of the electrochemical potential of sulphate ion is depicted in Figure 5. The critical pH induced increased sulphate permeability and consequently the sulphate electrochemical potential decreased toward the equilibrium. Inside-positive membrane potential in acidophiles may play some defensive role against cytoplasmic acidification and cation toxicity, but at the same time it may assist anion influx. In fact toxicity toward inorganic, monovalent anions in *T. ferrooxidans* has been shown to be due to their membrane-potential driven accumulation in the cytoplasm¹⁹. In *T. thiooxidans* protonated form of sulphite, sulphurous acid or sulphur dioxide has been reported to penetrate and acidify the cells²⁰. However, sulphate ion, having an appreciable dipole moment even in its fully protonated form, is likely to be impermeable to lipid bilayer. This is in agreement with our observation that sulphate was effectively excluded from the active cells. In fact, the electrochemical gradient of sulphate was sufficient to maintain a PMF of 110 mV over the pH range 1.8–4.0 (Figure 5). It is tempting to assume that an electrogenic sulphate transport system, perhaps directly coupled to proton translocation, plays some crucial role in bioenergetics of *T. ferrooxidans*.

So far, only limited interpretation is available to explain the characteristic pH profile of iron oxidation by

T. ferrooxidans. In this report, we propose a bioenergetic basis of its discontinuous decrease below the physiological pH. The cells appear to show increased anion permeability, leading to irreversible damage only under those situations where membrane potentials are expected to be very high. The cells were never found to possess a membrane potential value higher than 200 mV in equilibrium condition, which presumably represents a threshold. A plausible scheme is put forward to depict interplay between the chemiosmotic parameters and ion permeability in *T. ferrooxidans* (Figure 6). A transmembrane potential of 200 mV corresponds to an electrical field strength of about 300,000 V/cm. Such a high field may lead to dielectric breakdown – similar to non-ohmic leak/slip in state 4 mitochondrial respiration¹⁵. An increased fixed charge on the membrane surface, on the other hand, is also expected to affect membrane organization which in turn may lead to increased permeability. Our preliminary measurements of surface potential by spectroscopic probes suggest that near the physiological pH the surface potential is positive and increasing with decreasing pH¹⁸. Evaluation of the components of the membrane potential, viz. transmembrane potential and surface potential and their possible relation to the sulphate distribution, thus may lead to further elucidation of the bioenergetics and ion transport mechanisms in this organism.

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Visualization in biodiversity research: A case study of Mehao Wildlife Sanctuary, Arunachal Pradesh, north- east India

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Biodiversity visualization is a process by which diverse data sources varying from cover types, habitats to species inventories are integrated into a single computer environment. The integration affords a far better degree of spatial reasoning and understanding of biodiversity issues than hitherto attended to. We attempt here to integrate satellite data, digital elevation models derived from the toposheets, slope, aspect, elevation data and known information on some elements of biodiversity. The visualization tools were applied for a case study in Mehao Wildlife Sanctuary, north-east India, a global hotspot of biodiversity. The thematic features included in the visualization are habitats classified using the Landsat thematic mapper (TM) data, plant succession, hotspots of plant diversity, pheasant and takin habitat. The results were 'field tested' for acceptance among the user community. It is concluded that the visualization has a substantial role in furthering biodiversity conservation.

SCIENTIFIC visualization is defined as 'the study, development and use of graphic representation and supporting

techniques that facilitate visual communication of knowledge¹. The major objective of scientific visualization techniques is to process digital images to produce a simulated model leading to enhanced information content in the images². Biodiversity visualization is a means to achieve cognizance of spatial observations of biological diversity at many scales. This is usually achieved using computer-based scientific data visualization tools². Data visualization tools range from simple statistical bar charts to totally-animated realistic visual simulation and are intended to assist in the data-to-environment translation process. While this process of translating different states of environment into data and statistics exist, the converse of translating data to environmental images is less developed and often neglected³. The term biodiversity visualization denotes integration of diverse data sources on biological diversity – ranging from cover types, habitats, species inventories, birds and mammal vocalizations and so on into a single computer environment³. In the context of biodiversity research, the chief goal for data visualization lies in achieving accurate and verifiable representation of existing and projected environmental scenarios². The rationale for pursuing visualization is that it aids the scientist with better tools to grapple with the complex issues on patterns of distributions of species, ecosystems and landscape. The manager and policy makers likewise can readily comprehend the issues for day-to-day management. In this paper, we present a case study of data visualization in biodiversity research in north-eastern India, a globally recognized hotspot of biodiversity. The goal is to demonstrate use and integration of digital satellite data, digital elevation models, spatial terrain analyses such as slope, aspect and known information/data on vegetation, mammals and birds. We dem-

onstrate in particular the use of GIS overlay analyses to derive vegetation vulnerable to change, hotspots of plant diversity, pheasant habitats and habitats of rare and endangered mammal, takin.

The study area (28° to 28°15'N Lat., 95°45' to 95°50'E Long.) comprises 281.5 km² of lesser Himalayas of north-east India in the Dibang Valley district of Arunachal Pradesh. This area has been declared as wildlife sanctuary and is being managed for conservation of the unique values of biological diversity. Amongst the most notable features of this sanctuary is an altitudinal gradient spanning from a few hundred (500) to a few thousand (3300) metres of elevation, resulting in a variety of habitats, ecosystems and species. The habitats include tropical broad-leaved evergreen forests harbouring India's only ape, the Hoolock Gibbon and conifers in the higher altitudes, housing the endangered Red Panda. Blyths Trogon has recently been discovered in the sanctuary. Medicinal plants such as *Taxus baccata* and *Coptis teeta* occur and are extensively used by locals.

There are no published accounts on historical land use of this sanctuary. From the anthropological descriptions of various tribes and their customs⁴⁻⁶, it appears that there are no elaborate land laws nor any regulation of land use. The recent commercialization of timber from the forest gave a great impetus for both legal and illegal removal of timber from all accessible regions of the sanctuary. This is especially so in the lower (>1000 m) and mid (1000–2000 m) altitudes. The consequence of this exploitation has resulted in large-scale changes in vegetation. For example, it was observed that there is an increase in secondary vegetation and large-scale growth of bamboo. There seem to be discernible patterns of vegetation succession. For example, the cool northern aspects (the direction of slope, e.g. north, south, west, etc.) in the lower and mid-altitudes harbour *Musa* sp. and the southern aspects are often covered by bamboo and other deciduous vegetation. In the higher altitudes, the temperate evergreens dominate. Wherever disturbance is extensive, high altitude bamboo (*Schizostachyum* sp.) occurs widely. The alpine areas are located in altitudes exceeding 3200 m. The tree line is much closer (3000) to the alpine localities, compared to that of Western Himalayas.

Landsat TM digital data in bands 2, 3, 4 and 5 for 23 December 1993 were acquired for the study. The Survey of India (SOI) toposheets 91D04 and 82P16 covered the area of interest. Extensive field work in 1995 and 1996 provided the data for 'ground truth' and validation of remote sensing analyses and GIS modelling. The field work consisted of a) sampling tree, shrub and herb species composition at various altitudinal zones in 0.1 ha plots; b) sampling avian diversity in the same plots; c) obtaining ancillary information from the tribals on the distribution and abundance of pheasants and takin and; d) validation of the results.

For the entire study area, contours from SOI toposheets at 200 m interval were digitized in EASI/PACE environment on IBM RS6000 workstation at the Regional Remote Sensing Service Centre, Dehra Dun.

Digital Elevation Model (DEM) was created by the Morphology Dependent Interpolation Procedure (MDIP)⁷ in GRID interpolate (GRID INT) utility of EASI/PACE. Within GRID INT the CONIC option used as diagonal methods was found to be less satisfactory. The utility, PSGIMG (Image perspective) in Terrain Analysis module was used to obtain the terrain model from the DEM.

A rectification procedure for earth rotation effect in the satellite data for bands 2, 3, 4 and 5 was done using various utilities in the module of geometric correction. Necessary Ground Control Points (GCP) were taken from the SOI toposheets. An accuracy of 2 pixels only could be obtained as there have been substantial changes in river, road junctions and even the courses of river/streams. There are very few roads to specify precise GCPs. In terms of physical limits, the accuracy represents a ground area of 0.18 ha since each TM pixel is of 30 × 30 m size.

A False Colour Composite (FCC) was generated using bands 5, 4 and 3. The sanctuary boundary was digitized on to the rectified data and all further analyses were confined to this locality only.

The DEM was used in obtaining aspect (ASP) and slope (SLP) images of the sanctuary. Two utilities, ASP and SLP in the module of terrain analysis were used to obtain the above images. The two, along with elevation images, represent important habitat variables for biodiversity research.

The multi-layer modelling routine in EASI/PACE, permits a user-defined modelling approach. This involves specifying the precise nature of model and the computational procedure. GIS raster modelling was done to 1) locate hotspots of plant diversity; 2) vulnerable areas of vegetation change; 3) pheasant habitat suitability and 41 potential takin habitats.

The resulting images from the GIS raster modelling were draped onto the DTM in FLY! programme of EASI/PACE. The entire area was traversed at varying view angles, altitudes, magnification and at different locations. The resulting visualization afforded 1) better method of locating 'ground truth' samples for classification and 2) verifying the accuracy of various classification schemes adopted.

The false colour composite draped onto DTM is given in Figure 1. An unsupervised classification was done by *k*-means classifier. The classifier is essentially a clustering algorithm computing sample means through an iterative procedure⁷. The recruiting theme map provides each cluster with a specific grey value. A total of 36 spectral classes were obtained. With the help of ground

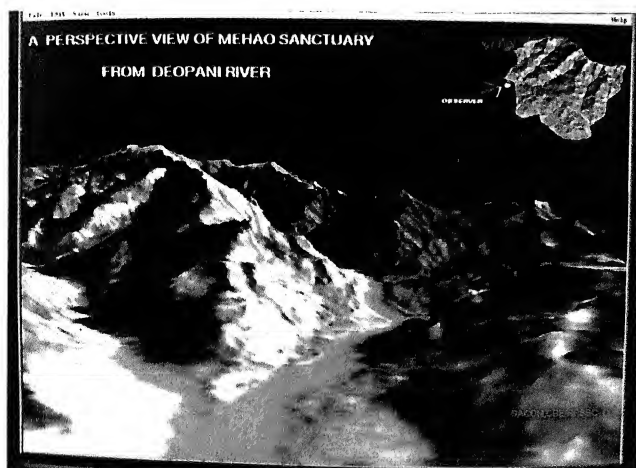


Figure 1. An FCC of TM bands 5, 4, 3 of Mehao Sanctuary, draped on Digital Terrain Model (DTM). The two rivers in the foreground are tributaries of Deopani river. The snow-capped mountains are at the rear. A two-dimensional FCC of the locality is in the inset.

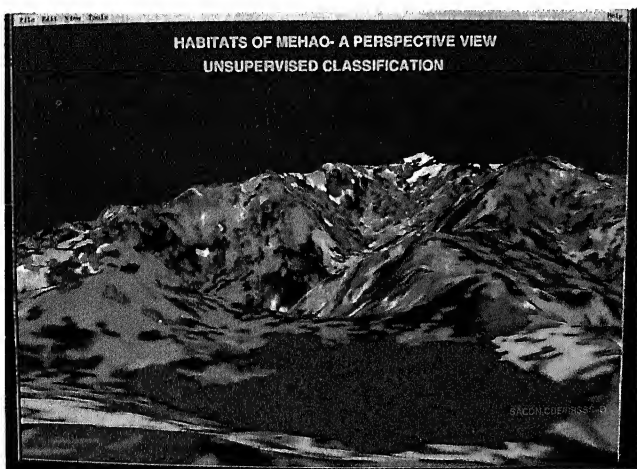


Figure 2. An unsupervised classification of TM bands 2, 3, 4, 5 gave broadly identifiable patterns of biomes. The various hues depict different spectral classes corresponding to habitat categories. In the foreground of the picture lies Mehao lake, a major landmark.

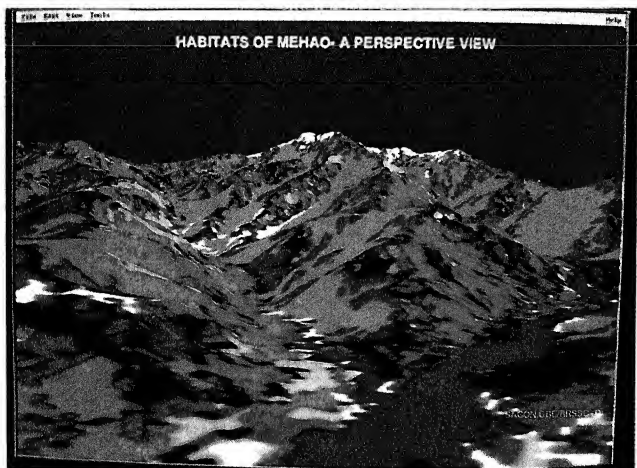


Figure 3. A supervised classification of TM bands 2, 3, 4, 5 resulted in the identification of eight major habitat categories including shades caused by high relief (shown in grey). The snow-capped mountain ridge is seen in the background.

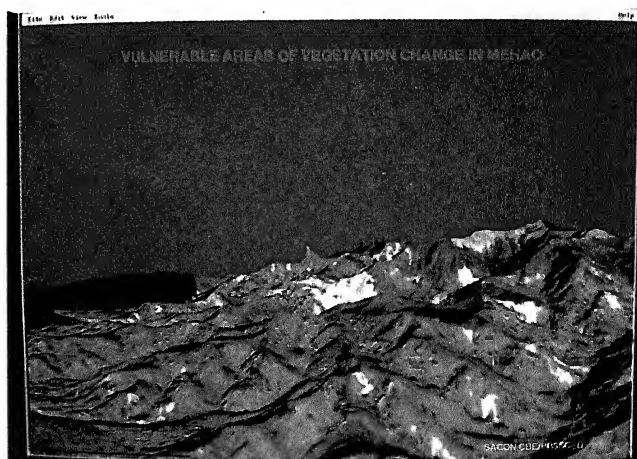


Figure 4. The thematic layers of habitat categories were analysed along with slope, aspect and elevation layers to identify vulnerable areas of vegetation changes.

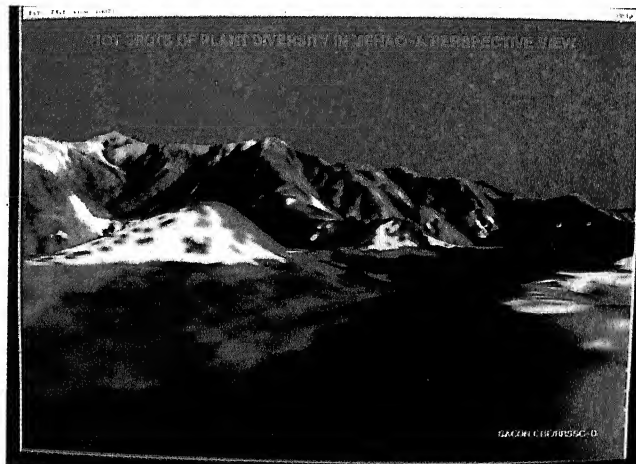


Figure 5. The results of field sampling on plant diversity were overlaid on physical attributes such as slope, aspect and elevation to obtain 'hotspots' of plant diversity. The yellow patches in the picture show these localities.

truth samples, these classes were later coalesced into 12 categories. The results were draped on DTM and are shown in Figure 2. There is abundant heterogeneity in the classes obtained. However, the high altitude habitats can be demarcated easily from the lower altitude habitats.

A supervised classification was done using maximum likelihood classifier. The accuracy of the classification was found to be only 80%. Again, a substantial extent of heterogeneity appears to contribute to the somewhat low accuracy. A thorough field check confirms the heterogeneity in small spatial unity. The results of supervised classification draped on DTM are shown in Figure 3.

The GIS models used were of necessity simple in nature. This is due to the fact that there are no ecological

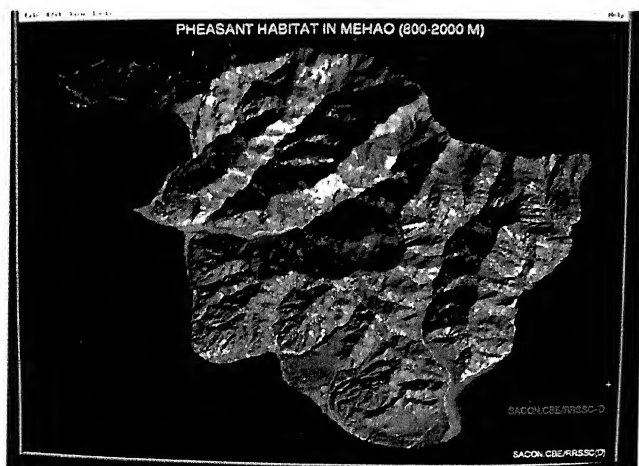


Figure 6. Pheasant habitats in the altitudinal range of 800–2000 m were identified and are shown in green colour.

and natural history studies in this area which could shed light on plausible models to be used. Since physical variables such as elevation, slope and aspect are important determinants of distribution of vegetation/habitat categories, these variables were used in four thematic features of relevance to biodiversity. It is to be pointed out that this is obviously an illustrative attempt and not comprehensive.

It is known that vegetation is vulnerable to change due to human activity. This is especially so in the south-facing accessible slopes ($<30^\circ$) with deciduous vegetation. Southern aspects by virtue of their longer duration of exposure to harbour, dry habitats with serial stages prone to change. In the overlay these conditions, e.g. deciduous vegetation on southern and south western aspects with slopes of less than 30° with altitude of less than 1800 m were specified. The percentage pixels qualifying these criteria was found to be only 2.83. The total area is 795 ha. However, spatially speaking, these locations are spread over the entire area. The results were draped onto the DTM (Figure 4).

From the field sampling on plant diversity, it appears that northern aspects with evergreen vegetation are likely candidate sites for hotspots. The hotspots are those that have highest species richness in the field samples. These were located by specifying masks for slope, aspect, elevation and vegetation themes. The mask size is 281.5 km². The percentage of pixels qualifying these conditions is 0.84. Hence, the total area under hotspots is 236 ha. A part of the hotspots draped on DTM is given in Figure 5.

This area is known for its pheasants. Blyth's tragopan, a highly endangered pheasant is routinely hunted. From the available information on habitat preferences of pheasants between 800 and 2000 m altitude, using the thematic covers of vegetation, slope, aspect, an overlay analysis indicated 1.4% of the geographical area

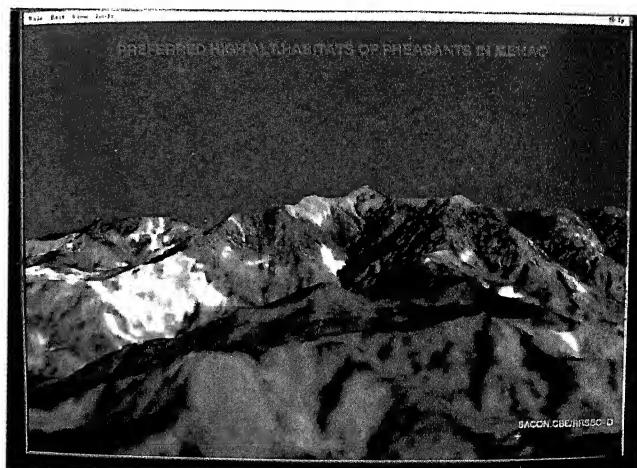


Figure 7. The preferred high altitudes of pheasants were identified using overlay analysis and the results were draped in DTM. The localities in green colour indicate these habitats.

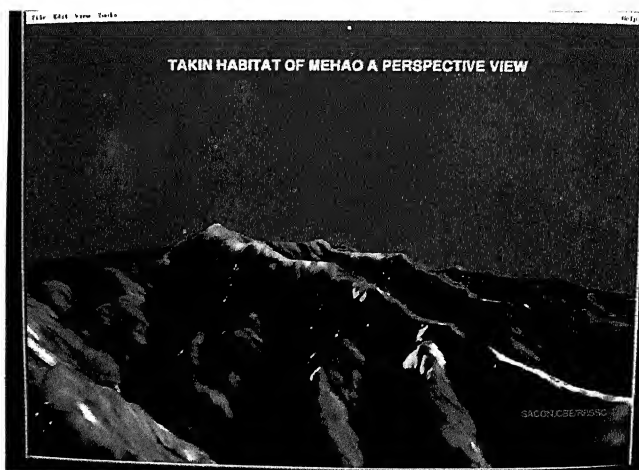


Figure 8. Takin, an endangered mammal, occurs in sparse numbers. The likely locality of occurrence are shown in yellow colour.

as suitable habitat. This works out to be 393 ha. The area is shown in Figure 6.

At higher altitudes (>2000 m), the winter habitat of pheasants is largely restricted to south-facing slopes. An overlay analysis using southern aspects with evergreen, semi-evergreen vegetation cover themes indicated 0.57% area as suitable habitat. This works out to be 150 ha spread over the entire area (Figure 7).

Takin, the largest representative of Rupicaprinae of the Bovidae inhabits some of the steepest mountains in mid-altitude tropical evergreen forests (90° –1200 m). It has a narrow range of global distribution, being confined to a few patches in Bhutan, part of China and parts of Arunachal Pradesh⁸.

Takin, is therefore a flagship species of biodiversity in Mehao and is an extremely rare mammal to be encountered. However, the hunters appear to hunt these animals

(which only points to the extent and depth of their knowledge on environment). From detailed description of the preferred habitat, using overlay analysis of slope, aspect, elevation and vegetation classes, we obtained the preferred habitats of Takin (Figure 8). The preferred habitat constitutes just 0.11% of the sanctuary. In real terms the extent is a mere 30 ha. This is possibly the major reason for the observed low encounter rates. It also points to the need of including available habitat outside of Mehao in a bigger viable conservation unit.

Visualization in biodiversity research entails a whole spectrum of potential benefits at the levels of species, ecosystem and at landscape. An explicit spatial dimension – in terms of patterns of distribution of the above three components of biodiversity, can be provided. This is especially useful in underexplored and relatively unknown field locations using simulated environmental images. The Rapid Biodiversity Assessment (RBA) procedures stand to gain immensely by using visualizing tools. As a sequel to RBA one could effectively use the visualization for evolving comprehensive and appropriate sampling designs for biodiversity description, assessment and monitoring.

For the routine natural history surveys of endangered rare species, one can use available information, howsoever meagre, along with visualization tools to focus the surveys in well-defined geographical locations. Such focused surveys of course, are not only cost effective but could contribute to better formulation of habitat suitability models for a number of species.

We thought it would be better to elicit reaction to these visualizations and perceived impressions on utility for field personnel. We have shown these and the two-dimensional photos to a wide group of people in the sanctuary. It was amazing to find the ease with which the people identified ground features draped on DTM. These persons could identify vegetational and terrain features much more easily and preferred the three-dimensional pictures.

The results on visualization can be altered/improved based on availability of further field data or models of habitat utilization of species of interest. Again, in the area of EIA studies, these visualization tools hold great promise in communicating the data in the form of environmental images to a wide group of users.

In conclusion, we hold the opinion that this methodology and its applications will find wider use in the coming years. This will undoubtedly go a long way in baseline information collection, assessing and monitoring biodiversity in ever-changing environment.

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Transient expression of β -glucuronidase reporter gene in embryogenic callus cultures of an elite indica basmati rice (*Oryza sativa* L.)

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Various parameters for the introduction of β -glucuronidase (GUS) reporter gene driven by actin-1 promoter into embryogenic callus cultures of an elite indica basmati rice cultivar (Basmati 370) through biolistic delivery method were studied. Helium gas pressure of 900 psi, arrangement of the callus at the centre of the plate or 1100 psi helium pressure and arrangement of the callus at the innermost concentric ring of the plate along with 6 cm distance from the microcarrier produced best results in terms of transient GUS expression, but with moderate callus survival. However, better survival of the callus with moderate GUS expression was observed when the callus was placed at the centre or the innermost concentric ring of plate with a distance of 9 and 6 cm using 900 and 1100 psi, respectively; and these parameters may be useful in recovering transgenics. These parameters will now be employed for the development of fertile transgenic basmati rice harbouring agronomically useful genes.

SIGNIFICANT progress has been made in the last two decades in the improvement of important cereals, such as rice, through conventional breeding methods.

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RESEARCH COMMUNICATIONS

However, application of genetic manipulation techniques is becoming increasingly important for rice improvement to cope with its growing need. Although, genetic transformation through *Agrobacterium* is very effective and a widely used method of obtaining transgenic plants in dicots, it is still a difficult proposition in monocots, including rice, though some success has been achieved in this respect, of late^{1,2}. Therefore, other methods of gene transfer such as particle gun, electroporation of polyethylene glycol-mediated transformation have been used for the achievement of fertile transgenic rice³⁻⁵ and other cereals^{6,7}. However, all of these reports have come from laboratories abroad. Certain elite indica rice cultivars, which are widely grown in our country, have not been used for these studies. Regeneration protocol from callus cultures in Basmati 370 which is an aromatic, fine-grained and highly-priced rice cultivar has been standardized in our laboratory⁸ with the aim of establishing transformation protocol in basmati rice. In this communication, we report, for the first time, the transient expression of β -glucuronidase (GUS) reporter gene through biolistic delivery in mature embryo-derived embryogenic callus cultures of rice (*Oryza sativa* L. cv. Basmati 370).

Mature seeds of rice were de-husked, surface-sterilized with 0.1% HgCl₂ for 20 min and then rinsed with several changes of sterile distilled water. For callus induction, the seeds were placed on Murashige and Skoog (MS)⁹ medium supplemented with 2 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and cultured in dark at 26 \pm 1°C. Following three weeks of culture, the callus induced contained both embryogenic as well as non-embryogenic regions. Embryogenic regions were excised and cultured on the same callus medium for another 2 weeks as described earlier¹⁰.

The PDS-1000 He biolistic particle delivery system (Bio-rad, USA) was employed in the present experiments and the microprojectiles were driven by helium pressure. The gene construct used was actin-1 promoter-actin 1 intron-gus-tnos (obtained from D. McElroy, CSIRO, Australia). Other details of this plasmid construct are published elsewhere¹¹.

The microcarriers (DNA-coated gold particles) were prepared by sterilizing 60 mg gold particles (Bio-rad, USA) with 1 ml of absolute ethanol in an eppendorf tube and kept at room temperature for 20 min. Following incubation, the particles were spun down and washed thrice with sterile distilled water and resuspended in 1 ml 50% glycerol. For DNA/particle precipitation, 12 μ g of plasmid DNA (DNA concentration 1 mg/ml) was added to 50 μ l of these particles, along with 50 μ l of 2.5 M CaCl₂ and 20 μ l of 5 M spermidine. Following vortexing and 20 min of precipitation at room temperature, the particles were spun down and resuspended in 140 μ l 70% ethanol. This procedure was repeated once with 40 μ l ethanol. Finally, 6 μ l of the suspension was used per shot.

Table 1. Bombardment conditions employed in the present investigation on basmati indica rice callus

Distance between rupture disc and macrocarrier	0.5 inch + 15 mm
Size of gold particles	1 μ m
Distance between microcarrier and target	6 and 9 cm
Density of particles/shot	9 mg/shot
Bombardment medium	MS medium supplemented with 2 mg/l, 2,4-D (rice callus medium)
Callus placement in the petri plate	In the centre and in various concentric rings in 90 mm diameter petri plate
DNA concentration	1 mg/ml
Physical parameters:	
Helium pressure	450–1300 psi
Vacuum	26 mm Hg
Post-bombardment culture	On callus medium till GUS assay (48 h)

Table 2. Transient expression of GUS at various arrangements of rice callus at the time of bombardment with variable helium pressure

Helium pressure (psi)	Arrangement of callus in the petri plate	Transient expression of GUS*	Callus survival†
450	At the centre (distance between microcarrier and the target = 6 cm)	–	Good
650	At the centre (distance = 6 cm)	+	Good
900	At the centre (distance = 6 cm)	+++	Moderate
	At the centre (distance = 9 cm)	++	Good
	At the innermost concentric ring of the petri plate (distance = 6 cm)	++	Good
	At the innermost concentric ring of the petri plate (distance = 9 cm)	+	Good
1100	At the centre (distance = 6 cm)	++	Died
	At the centre (distance = 9 cm)	++	Moderate
	At the innermost concentric ring of the petri plate (distance = 6 cm)	++	Good
	At the innermost concentric ring of the petri plate (distance = 9 cm)	+	Good
1300	At the centre (distance = 9 cm)	–	Died‡

*Based on the distribution of blue spots on the callus; +, poor expression; ++, moderate expression; and +++, good expression of GUS; †Callus survival was observed in 15-day-old cultures; ‡Callus died within 48 h of culture.

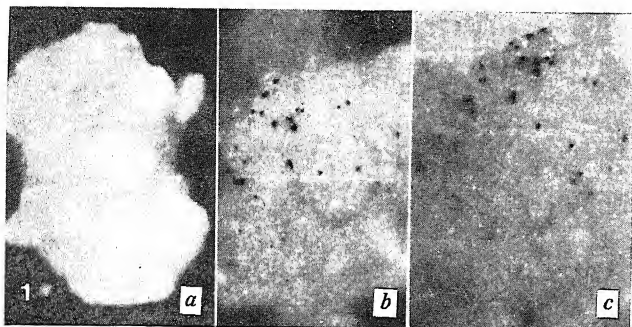


Figure 1. Transient expression of GUS in bombarded callus of rice arranged in the centre of the plate with a distance of 6 cm and 900 psi helium pressure. Non-bombarded callus (a) and GUS expression in bombarded callus (b and c).



Figure 2. Arrangement of the callus at the innermost concentric ring of the plate on the platform for bombardment.

The target tissue (embryogenic callus) was arranged in concentric rings in sterile petridishes (90 mm diameter). The other physical parameters employed for the use of particle gun are summarized in Table 1. After the bombardment, the calli were cultured in dark for 48 h after which GUS staining was performed at 37°C as described by Jefferson¹².

Various parameters were employed for optimizing conditions for bombardment of rice callus to get transient GUS expression (Table 1). The lower helium pressures (450–600 psi) proved to be suboptimal while higher pressures (900 and 1100 psi) produced optimum results as evident in the form of blue spots on the callus following GUS histochemical assay (Figure 1; Table 2); GUS expression was not detected in control, non-bombarded callus (Figure 1 a). However, the highest helium pressure (1300 psi) led to the complete browning

of the callus (Table 2). Therefore, further optimizations were carried out using 900 and 1100 psi helium pressures.

Subsequently, the arrangement of the callus on the concentric rings of the plate was standardized (Table 2). First, the callus was placed at the centre followed by placing of the callus in the two innermost concentric rings. The maximum expression of GUS, concomitant with moderate survival of callus, was observed when the callus was placed at the centre or the innermost concentric ring of plate with a distance of 6 cm using 900 and 1100 psi helium pressure, respectively (Figure 1 b and c; Figure 2). However, better survival of the callus with moderate GUS expression was observed when the callus was placed at the centre or the innermost concentric ring of the petri plate on the platform at a distance of 9 and 6 cm using 900 and 1100 psi, respectively (Table 2). The latter parameters may be useful in recovering transgenics. These standardized bombardment conditions will be utilized for the development of fertile transgenic basmati rice harbouring agronomically-useful genes.

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Impact of magnesium-aspartate hydrochloride on glucoregulatory system of two avian species

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Chronic administration of magnesium aspartate hydrochloride (Mg-Asp.HCl) induced hyperglycemia, hypoinsulinemia and hypercalcemia in common myna. But there were no changes in blood sugar and plasma insulin levels in the magnesium-fed pigeon. Plasma magnesium markedly increased in both the avian species, but plasma calcium level decreased significantly in pigeon, unlike common myna. Magnesium had no effect on pancreatic glutathione concentration in both pigeon and common myna. Concentration of magnesium and/or food habit may be the probable two factor(s) responsible for the different glycemic responses in pigeon and common myna.

THE interrelationship between magnesium and carbohydrate metabolism has regained considerable interest over the last few years. Insulin secretion requires magnesium. Magnesium deficiency results in impaired insulin secretion, while magnesium replacement restores insulin secretion. Experimental magnesium deficiency reduces the tissue sensitivity to insulin¹. Paolisso and Ravussin² reported that intracellular magnesium acts as a cofactor for numerous enzymes involved in carbohydrate metabolism. Despite the reports in mammals, except for chicken³, very little is known about the role of magnesium on the glucoregulatory mechanism of birds. In rats, depending upon the dose administered, magnesium-aspartate hydrochloride (Mg-Asp.HCl) exhibits Ca-antagonistic effect and inhibits the release of stress hormones^{4,5}. In chickens, changes in plasma level of magnesium have been reported to cause alterations of the rate of insulin release³.

The paper ascertains the role of Mg-Asp.HCl on gluco-regulatory mechanism with special reference to insulin release in two avian species belonging to different phylogenetic and nutritional status, and aims to establish, whether the response is related to the latter factor. Mg-Asp.HCl was used, since it is readily absorbed through the intestine⁵.

Adult specimens of pigeon (*Columba livia*) and common myna (*Acridotheres tristis*) were acclimatized to laboratory condition for a week. The animals were starved 18 h prior to experimentation, though water was provided *ad libitum*. Mg-Asp.HCl was orally adminis-

tered to pigeon (600 mg/bird/day) and common myna (300 mg/bird/day) in the morning at 10 a.m. for six consecutive days. On the 7th day, prior to autopsy, blood was withdrawn from the wing vein for biochemical assays. Birds were autopsied by cervical dislocation. The pancreatic tissues were dissected out and fixed in chrome-alum-Bouin's fixative⁶ for routine microtomy and 5 µm thick paraffin sections were stained using Eple's⁷ aldehyde fuchsin trichrome method. Using arsenomolybdate colour reagent, blood sugar was estimated by Nelson and Somogyi's method. Plasma insulin was measured by a direct radioimmunoassay with protocol and radiochemicals supplied by Board of Radiation and Isotope Technology, Mumbai, India. The radioactivity was measured in LKB-γ counter. Calcium was measured by using methylthymol blue method of Glindler and King and colour was measured in Perkin Elmer spectrophotometer (Model 550 S, USA). Magnesium was determined by the fluorometric method of Schachter and fluorescence was measured by Fluorescence Spectrophotometer (Hitachi, Model 650, Japan). Splenic lobe tissues of pancreas were collected and glutathione was estimated using spectrofluorometric method of Hissin and Hilf⁸. The statistical analysis was carried out by Student's *t*-test.

The islets of Langerhans are distributed more or less uniformly throughout the entire pancreas. Islets are composed of A, B and D cells. A cells are large with well marked outlines. The nuclei are round and oval with prominent nucleoli. The B cells consist of deep purple aldehyde fuchsin positive granules. The D cells are mostly spindle-shaped and are smaller than A and B cells. The D cell nuclei fill up the entire cell surface and possess light green positive cytoplasm. But no significant changes were observed in the islet cytology under light microscope after chronic treatment of oral Mg-Asp.HCl, both in pigeon and common myna.

A marked increase in blood sugar level and decrease in plasma insulin concentration were noticed in the magnesium-treated common myna. Blood sugar and plasma insulin levels remained unchanged in magnesium-fed pigeon. Plasma magnesium level significantly increased in both the experimental birds. Plasma calcium level significantly decreased in pigeon whereas in common myna its concentration showed a marked elevation. Pancreatic glutathione was not altered after treatment in both pigeon and common myna (Table 1).

An earlier report showed that disturbances in $\text{Ca}^{2+}/\text{Mg}^{2+}$ ratio alter the B cell responsiveness³. It is known in mammals that the intracellular calcium is essential for insulin release, while magnesium inhibits Ca^{2+} uptake⁵. Thus the increase or decrease of plasma calcium level is expected to increase or decrease the insulin release. In the present investigation, chronic treatment of Mg-Asp.HCl produced hyperglycemia, hypoinsulinemia and hypercalcemia in common myna. The

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Table 1. Changes in blood sugar, plasma insulin, plasma calcium, magnesium and pancreatic glutathione after chronic treatment (6 days) with Mg-Asp.HCl in pigeon (*Columba livia*) and common myna (*Acridotheres tristis*)

Species	Dose	Group	Blood sugar (mg %)	Insulin (μ U/ml)	Calcium (mM/l)	Magnesium (mM/l)	Glutathione (mM/g)
<i>Columba livia</i>	600 mg/bird/day	Control	185.14 \pm 9.24 (7)*	9.06 \pm 0.54 (7)	3.27 \pm 0.13 (7)	0.67 \pm 0.06 (7)	8.08 \pm 1.03 (7)
		Treatment	192.85 \pm 6.62 (8)	8.88 \pm 0.52 (8)	2.60 \pm 0.20 (9)	1.24 \pm 0.09 (7)	7.94 \pm 2.11 (7)
			NS	NS	p < 0.005	p < 0.001	NS
<i>Acridotheres tristis</i>	300 mg/bird/day	Control	200.21 \pm 11.44 (7)	27.18 \pm 2.92 (7)	3.19 \pm 0.14 (7)	0.36 \pm 0.03 (7)	9.64 \pm 1.42 (7)
		Treatment	250.31 \pm 18.03 (7)	15.52 \pm 0.84 (7)	4.27 \pm 0.13 (7)	0.40 \pm 0.0009 (7)	10.45 \pm 0.94 (7)
			p < 0.05	p < 0.005	p < 0.001	p < 0.001	NS

*Mean \pm SE; NS = Not significant.

Figures in parenthesis represent the number of birds.

earlier report had shown that both Ca^{2+} and Mg^{2+} ions compete for a common cationic carrier system located in the B cell plasma membrane⁹. Hence, in the common myna, magnesium probably inhibits glucose-induced calcium uptake and subsequent insulin release by the B cell. Similar findings are also reported in mammals¹⁰. Our finding is supported by the observation of Hazelwood¹¹ in chicken where absence of magnesium in the perfusate markedly increases insulin output. In contrast to common myna, pigeon exhibited no significant changes in blood sugar and plasma insulin levels after chronic treatment of magnesium. Plasma level also decreased significantly in pigeon as compared to common myna. Hence, our results indicate that pigeon deviates from common myna and chicken¹¹, in its glucoregulatory mechanism after chronic treatment with magnesium. Classen *et al.*¹² pointed out that concentration of Mg-Asp.HCl is very important in exhibiting its Ca-antagonistic action. Thus, it is quite possible that the magnesium concentration was not optimum to cause Ca-antagonistic action and in turn failed to alter insulin release in pigeon, unlike in common myna. The differential findings between the two avian species could be due to the different nutritional status of pigeon and common myna which support the earlier work of Bentley¹³. Furthermore, increased level of plasma magnesium has no effect on pancreatic glutathione content in the two avian species studied.

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BOOK REVIEWS

Stereochemistry and Mechanism through Solved Problems. P. S. Kalsi. New Age International Publishers, 4835/24, Ansari Road, Darya Ganj, New Delhi 110 002. 1994. x + 373 pp. Price: Rs 165.

The book deals with the topics stereochemistry and reaction mechanism through problems and solutions. The purpose of the book as suggested by the author in the preface, is to train students for appearing in post-graduate university examinations as well as competitive examinations like joint UGC-CSIR eligibility test. The book therefore, serves as a model question-answer book for strategic learning.

Both the areas of application and strategic learning are most sought-after materials for the benefit of the students. The book is useful to teachers for selecting problems for discussion in the classroom, setting questions for formal and terminal tests. The purpose of the book is to train students in application of the knowledge and understanding they have in stereochemistry and reaction mechanism. Problem solving builds up the application aspect of learning. The book presents each subtopic through posing a problem and solving it. Therefore, this book provides a very good medium for self-learning the application of knowledge in the area of stereochemistry and reaction mechanism.

The book contains eleven chapters. Each chapter begins with enlisting salient features of subtopic, followed by problems and solutions in the form of model answers, gradually covering the entire subtopic. The book is full of nice illustrations which depict three-dimensional aspects of the molecular structures. Chapter 4 develops the fundamental concept of molecular symmetry and chirality which is very helpful in learning stereochemistry.

Chapter 6 deals with Optical Rotatory Dispersion as an instrumental method for determining configuration of some molecules. Problem 1.60 of Chapter 1 also discusses use of NMR in identifying certain conformers. However, a separate chapter discussing principles of instrumental methods and their application to identify stereoisomers and to determine three-dimensional molecular structures would

have been useful to readers.

It will be beneficial to the readers if aliphatic electrophilic substitution reaction (SE1, SE2, and SEi) and the effect of substrate, leaving group and solvent can be explained with examples in a separate chapter. Again, if the reaction intermediates, i.e. carbonium ions (classical and non-classical), carbanions, carbenes, nitrenes and arynes are discussed elaborately with examples, it will be helpful to the students appearing at various competitive examinations.

The book needs minor corrections at some places.

Some questions do not contain proper action verbs. For example: Problem 1.5 (page 5) states 'Draw and explain the hybridizations...', which can be modified more appropriately as 'Explain the hybridization... with suitable diagrams'. Problem 1.23 (page 16) stating: 'What do you understand by gauche and anti-conformations' may be modified as 'Define gauche and anti-conformations'. Problem 4.13 b (page 139) stating 'Tell, if *trans*-1,2-dichloro cyclopropane is asymmetric or dissymmetric?' can be modified as 'State whether *trans*-1,2-dichlorocyclopropane is asymmetric or dissymmetric'.

Some answers are not explicit, some are with mistakes. For example, in problem 1.85 (page 48) numbering of carbons in cubane would have made it easy for a reader to correlate the nomenclature. In problem 2.71, the answer states the condition for a compound to be meso isomer whereas the question was to identify a meso isomer in a list of three compounds. In problem 2.72, the answer does not explain why only three isomers are observed when there is a possibility of four isomers. Besides, the diagram of *cis*-1,3 dimethylcyclohexane would have made the answer complete. In Chapter 10, Huckel and Mobius systems should have been explained with diagram showing nodes.

In problem 1.25 and many others, the answer is in the conversational style. The book aims at giving model answers for written tests. So the answer should not be a phrase or an incomplete sentence.

The order of some problems needs change. For example, answers to problems 8.6b and 8.6c are referred to Chapter 9 (9.33a and 9.33b). The ques-

tion of stereo and regio selectivity should not have been raised in Chapter 8 when it is discussed in Chapter 9.

As a whole the book is very useful to both teachers and students for classroom and test purposes.

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State of the Art in Polymer Science and Engineering in India. M. Santappa. T. R. Publications Pvt. Ltd., PMG Complex, 2nd Floor, 57, South Usman Road, T-nagar, Chennai 600 017. 576 pp. Price: Rs 795.00

In this book the author has compiled the summary (often in one line) of the research papers on polymers published by Indian scientists, technologists and engineers based on their research work carried out in India for the last fifty years or so. He has set aside biopolymers excepting collagen from the purview of his compilation and also the polymer involved works in the fields of electronics, material science and space science. The author has, by and large, refrained from making commentary on the works and accordingly the book turns out to be a reference book on the subject and not a review. In the preface of the book he has commented that although our contributions to the polymer literature are substantial in quantity, they are poor in quality with very few exceptions, of course. Our works are mostly repetitive, uninteresting and often devoid of a clear objective. The question therefore arises - Why then did the author undertake such an arduous task to compile such voluminous but insignificant contributions? The author answers that the book has laid bare the glaring deficiency in the quality of our research papers. It comes as a lesson for the posterity so that quality gets priority over quantity.

The author is one of the seniormost and a much respected member of the profession. So his comments command attention and respect. But is the scenario not the same for every other field of research in our country? That this is

so is common knowledge. In order to find the cause behind the generally dismal performance in scientific research, one has to look into the socio-economic conditions of the country. We are amongst the poorest nations of the world with an average per capita income of around 300 dollars and a literacy rate of only about 50%. With such abysmally low economic condition and literacy it is hard to expect that our science will flourish and the general level will compare with the best in the world. It may be possible to build certain centers of excellence with excessive funding for the sake of improving the quality of research. But this would not help improve the general level, as our experience during the last two decades of effort in this respect would prove.

It may be pertinent here to discuss the situation in the two so-called Asian tiger countries, viz. South Korea and Taiwan. There were hardly any publications coming out from these countries even 20 years ago. Now, they are economically sound and scientific research there has gained ground so much that our scientists are flocking to these countries to gain post-doctoral research experience. Paradoxically, according to the *Economist* (Economist Newspaper Limited, London, 1996 reproduced in the *Sunday Statesman*, Calcutta, 22 September, 1996) one of the key factors contributing to the great economic boom in these countries has been identified to be their emphasis in imparting primary as well as secondary-level education to the whole population rather than to a part of it which would otherwise have assumed elitist character. The university education and research gained importance in a subsequent stage. The example of these two countries which belonged to the third world three decades ago proved right the prophecy of Carl Sagan, the renowned astrophysicist, who holds the view that Science in the third world countries is not gaining ground because of the lopsided educational policy which is elitist in character. We are yet to learn from the above examples.

Coming back to the book proper, the author has done a stupendous job in going through so many papers, which amount to more than one thousand. He has classified the work into eight top-

ics, covered in as many chapters. These are; 1. Synthesis of novel monomers, catalysts and polymers 2. Kinetics of polymerization; 3. Graft copolymerization; 4. Physical chemistry of polymers; 5. Chemical engineering and technology of polymers; 6. Some properties and uses of polymers; 7. Applications of polymers in industries; 8. Science & Technology of leather. The last one surely has found place because of the association of the author with the Central Leather Research Institute as its Director. The contributions in each of the above topics have been entered institutionwise, i.e. Universities, Research institutes, CSIR laboratories, IITs, other technological Institutes, Defence Research Laboratories, Space and atomic research laboratories. Also as has been stated earlier, the author has not made any commentary on the works except on rare occasions. Accordingly, the book is not a review but a dictionary of polymer research done in India during approximately the last 50 years.

It may be noted that the bulk of polymer research in India consisted of studies on free radical polymerization, finding out new initiator systems and establishing the kinetics and mechanism of polymerization reactions. In many other countries, polymer research started in much the same way. But while they quickly caught up with the ever-emerging new lines of research, we in India continued in the same line far too long. One obvious reason is that this particular line of research required minimum of funding. At present, however, in some of our laboratories research is going on in contemporary topics but these are very few in number. Unfortunately, on going through the book one does not easily get a feel of this change.

Furthermore, even though the stated policy of the author is not to evaluate the merit of the works, it would have been proper to highlight the landmark achievements which are not many in number and discuss them in brief. Take for example, the dye techniques developed by Palit in the early sixties. This technique stands out as the most original work from India in the sixties. It provided the polymer chemists with an easy and inexpensive means of identifying transient free radical intermediates which are used to initiate free radi-

cal polymerization. The technique served as a substitute of the hazardous radio-chemical assay technique. Using this, Palit and coworkers unraveled the mechanism of a large number of redox reactions that generate free radicals. Although mention has been made of this technique on pages 71 and 139, the significance and importance of the technique have not been properly made out and a brief description of the principle of the technique would have been desirable. This is particularly so since the author has in many places of the book devoted space to describe non-seminal works. The result is that many a gem got lost in the milieu.

As regards the preparation of the book, the compilation is disjointed in many places. Consider, for example, the two sentences under section 6 on page 139. The first sentence speaks of Palit's dye technique while the immediately next one deals with an entirely different subject. Also wrong references are cited for several works. For example, on page 70, 3rd para, refs. of Maiti *et al.* and Kar *et al.* are wrongly placed, similar is the situation with Das *et al.* in para 4 and also the references 147-151 in the same para are inappropriate. Furthermore, some references are also factually wrong, e.g. 166 and 167 on page 92. In chapter 1 there is no mention of the contribution from Indian Association for the Cultivation of Science in the contents and this contribution has been included under that of Calcutta University on page 27, section 10. There is also no uniformity maintained in the reference list. On the surface these lapses do not appear to be grave enough but such errors do not befit a reference book.

Finally, the title of the book *State of the Art* seems a misnomer. Perhaps, an appropriate title would have been 'A Concise Account of Research of Polymer Science and Engineering done in India' or something similar. Nevertheless, the book will serve the purpose of a source book on the subject and like all books of reference it should find place in technical and science libraries.

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A pioneer in X-ray crystallography, electron microscopy and biophysics

An obituary of R.W.G. Wyckoff (1897–1995)

Ralph Walter Graystone Wyckoff, one of the most remarkable American men of science, died at the age of 97 in 1995. A study of his life gives us some glimpses into the early history and developments of X-ray crystallography, electron microscopy and biophysics in USA.

Wyckoff's forebears came from Holland and settled in 1634 in the city of New Amsterdam which is now called New York. His father was a buildings contractor and Ralph was the first scientist in his family. Even when in school, he published papers in the *Journal of the American Chemical Society* and also took some patents. As a graduate student in Cornell University he made a serious but unsuccessful attempt to discover the missing element eka caesium from *pellucite*, a caesium containing mineral. This element, a short-lived radioactive one, was later discovered by Mlle Peres, one of Madam Curie's collaborators. While searching for a new problem for his doctoral thesis, Wyckoff came to know that in Cornell there were two visiting Japanese scientists – Nishikawa and Asahara. Shoji Nishikawa was a student of the famous Tarahiko Tarada (1878–1935) who discovered 'Bragg's' law independently of Bragg. As early as 1913 Nishikawa discovered X-ray fiber diffraction patterns of textile materials and of asbestos and did the structure of spinel. He was the first to realize the importance of the theory of space groups as a general and logical means for X-ray analysis.

Wyckoff, under the direction of Nishikawa, solved the structure of caesium dichloriodide and sodium nitrite which were amongst the earliest structures to be solved in USA using single crystal diffraction. He used Laue photography – but soon shifted over to photographing Bragg reflections. After his doctorate and immediately after the end of the war, Wyckoff went to the Geophysical Laboratory, Washington where he set up an X-ray group to determine inorganic and minerals structures. In 1922 he published the famous tome – *The Analytical Expression of the Results of Space Groups as Applicable to the Calculation of the Structure Fac-*

tors. This book went into several editions and was responsible to a large extent in the spurt of the activity in X-ray crystal structure analysis. About the same time, many others including Kathleen Lonsdale in England and Hermann Mauguin in France derived similar expressions. These, together with those of Wyckoff were all incorporated in the International Tables published by the International Union of Crystallography.

When the physical chemist Noyes went from MIT to the California Institute of Technology (Caltech), he was intent on starting X-ray crystallography as he felt that determining the positions of atoms in a crystal would be very im-



portant to chemistry. Ellis, who was to be in charge of X-ray crystallography development in Caltech came to Wyckoff's laboratory to familiarize himself with the subject. Wyckoff himself also went to Caltech to help set up the X-ray laboratory under Dickinson – with whom Linus Pauling was to work and create a major revolution in the field of X-ray crystallography, inorganic and mineral structures.

In 1927 Wyckoff went to the Rockefeller Institute for Medical Research in New York and started a new laboratory to investigate organic structures. Robert Corey trained in Wyckoff's laboratory on organic crystals, solved the structure of urea there and then moved over to Caltech and became known for his work on amino acids, etc.

Wyckoff's interest in crystals of biological interest was fostered by his close associations with P. Lecomte du Nouy, one of the pioneers of biophysics

and with Alexis Carrel whose development of tissue culture made it one of the fundamental techniques of modern biology.

Wyckoff (and his collaborators) attempted to crystallize proteins to obtain their X-ray diffraction patterns. He felt that the ultra centrifuge could be used for purifying proteins. As a first step to preparing good crystals, he went on to design and construct comparatively large ultra centrifuges with airturbines. In his design he substituted the steels normally used in such instruments by light and strong aluminum-magnesium structural alloys developed by Dow Chemicals. He used these centrifuges for purification of plant and animal proteins and viruses and obtained extremely beautiful protein crystals including that of haemoglobin. Unfortunately he did not succeed in getting X-ray diffraction patterns (probably because he did not keep the crystals in their mother liquor during photography as Bernal and Hodgkin did later).

He then went on to newer applied fields. Equine encephalomyelitis virus was the first of the viruses to be purified by this method. Wyckoff and J.W. Beard made an effective formaline-killed vaccine from these purified preparations. In view of the serious encephalomyelitis epidemic among horses in the United States, Wyckoff was invited to work in the Lederle Laboratories, where he developed, tested and produced several million doses of this vaccine. Following their massive use, the epidemic ceased and there was no major recurrence of the disease in USA. Wyckoff's laboratory then turned to the production of vaccine for typhus fever which was then raging. Large quantities of vaccine were made for the use of the US army. In response to the need for human blood plasma for treatment of war wounds in the Second World War, Wyckoff and his group next developed the process and built large-scale freeze drying plants for plasma with which they produced 2000 plasma packages per day.

After this very successful detour into applied and useful biophysics, Wyckoff returned to the life of fundamental research in the University of Michigan.

Because of his manual dexterity, he was able to repair an old and unusable electron microscope and in this process he suggested so many improvements that he was appointed consultant to RCA, USA and Phillips, Holland. During his electron microscopy researches, Wyckoff had difficulty in estimating the size and height of virus particles. At that time he met Robley Williams, a Caltech astronomer who was coating some mirrors in the Dept of Physics and Astronomy in Michigan. Williams said that it was standard practice to get the height of lunar mountains by knowing the angle at which the sunlight falls on them and measuring the length of the shadows they cast. The solution was to make the viruses cast shadows by evaporating gold from one side and leaving a shadow on the far side. The technique of metal shadowing, so familiar to electron microscopists now, was one of the major contributions of Wyckoff and his group to electron microscopy.

For the first time the shadowed virus particle revealed its true three-dimensional shape and photographs of particles of several viruses were taken. A particularly satisfactory development that followed was the direct demonstration of the ordered arrangement of molecules within 'virus crystals'. So impressed was J. D. Bernal with these photographs and developments that he invited Wyckoff to an international meeting he was convening in London. The participants were amazed at the simplicity and effectiveness of the technique. It was after this meeting that shadowing became a universal tool in the hands of electron microscopists.

It was also after this International meeting that the early confabulations took place between W. L. Bragg, P. P. Ewald, J. M. Bijvoet, R. W. G. Wyckoff and many others about the starting of the International Union of Crystallography and the International Union of Electron Microscopy. The former was established in 1948 with W. L. Bragg as its first President, J. M. Bijvoet its second (1951) and R. W. G. Wyckoff its third (1954). The International Union of Electron Microscopy was formed much later. When Wyckoff was at the Rockefeller Centre, he had started looking at collagen and later did much work on the electronmicroscopy of collagen

in the National Institute of Health and other institutions in Washington where he went from Michigan. His old friend Nishikawa sent one of his students H. Noda to work with him. Noda went back to Japan and became one of the authorities on collagen and its fine structure.

After retirement, Wyckoff joined the University of Arizona and set up a laboratory for the study of the fine structure of biological solids. Here he extended his interest in collagen to other proteins. He was amongst the first to find collagen in fossilized bones of the animals of Pleistocene era. His laboratory went on to study older fossils and dinosaur bones. He also used paper and liquid chromatography for the analysis of the amino acids extracted from these bones. Using electron microscopy he studied the structures of proteins in fossilized bones and proved beyond any doubt that collagen can persist over the geologically significant periods of time. His laboratory also analysed the ancient proteins, especially collagen, to see how they differ from the present day ones and whether these differences are due to chemical and/or biological evolution.

One of Wyckoff's life-long projects was the unified description of the results of crystal structure analysis. This resulted in the publication of his first group of volumes on the crystal structures (4 sections and 5 supplements, 1948-1960) and his later *Crystal Structures* (5 volumes, 1963-1967). He had put in an enormous effort into this but as it has been said 'his effort was much less and that he saved for thousands of crystallographers later'.

Wyckoff was recipient of many honours. He was elected to the National Academy of Sciences, The American Academy of Arts and Sciences and foreign member of the Royal Society, London and Royal Academy of Netherlands and a corresponding member to the Academie des Sciences, Paris. He was very proud of his election as Honorary Fellow of the Indian Academy of Sciences (1955). Almost every country in the world honoured him for his work on X-ray crystallography and electron microscopy. The American nation was grateful to him for his production of life-saving vaccines for which he was awarded many prizes.

I met R. W. G. Wyckoff in Washington in the spring of 1955. I first congratulated him on his election as President of IUCr. He said he cherished his election as Honorary Fellow of the Indian Academy of Sciences more. The reason he gave was that his attachment and ambitions in crystallography were not as much as his admiration for Indian philosophy, especially that of Ramakrishna Paramahansa and Swami Vivekananda.

Wyckoff knew C. V. Raman well ('highly original guy and a bit too energetic'). They had met first in 1924 when Raman had gone to Caltech as a visiting professor and again later in 1948 in Boston. Raman had bought for his personal library all of Wyckoff's books immediately after they were published, e.g. *Structure Factors* in 1922 and *Crystal Structures* in 1924. Wyckoff happened to be the examiner of my D Sc thesis. He was not at all happy that I had not accepted the fellowship he had offered me to work with him in electron microscopy. ('You missed a lot as you were not there to participate in the exciting things that were happening'). I remarked about his work on the dispersion corrections in anomalous scattering and on the later epoch-making discovery of Nishikawa and Matsukawa of the failure of the Friedel's Law under anomalous scattering conditions, on both of which some of my X-ray work was based. He talked most affectionately about Nishikawa and his association and life-long relationship with him. After the second world war Wyckoff resumed close contacts with him which were unbroken till Nishikawa's death in 1952. 'I am eternally grateful to him for introducing me to Japanese culture, Japanese art and to the eastern philosophies.'

Wyckoff did much for the growth of X-ray crystallography internationally and in USA. To quote from one of the articles in *The 50 years of X-ray Diffraction* edited by P. P. Ewald - 'The meeting of Ralph Wyckoff with the shy and gentle Nishikawa in 1917 may be said to have an implicit significance to the growth of X-ray crystallography in USA'.

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GOVERNMENT OF INDIA

DEPARTMENT OF SCIENCE & TECHNOLOGY

Programme on Nonlinear Phenomena, Complex Matter and Biologically Inspired Physics

The importance of nonlinear science, soft condensed matter (such as colloids, powders, polymers and membranes) and biologically inspired physics has emerged very clearly in recent years. In view of this and given the large mutual overlaps of these disciplines as well as their interdisciplinary nature, the DST has considered it fit to constitute a separate Programme Advisory Committee (PAC) specifically to foster research in these areas. Suitable project proposals are invited in the above general fields.

Research proposals should pay particular attention to the development and application of ideas and techniques that cut across disciplines and/or seek to elucidate and provide insight into the general paradigms of complexity and nonlinearity.

Proposals that come under this general purview include:

Dynamical phenomena far from equilibrium, driven systems, chaos, especially spatiotemporal. This encompasses both model systems such as sandpiles and driven lattice gases as well as 'real' examples such as colloids in shear flow and sedimentation, turbulence, kinetics of phase ordering, granular matter (powders), earthquakes, pattern formation, etc.

Physics of soft condensed matter. Membranes (biological and model), Langmuir monolayers and Langmuir-Blodgett films, micelles, vesicles, lamellar structures, microemulsions, emulsions, foams, and other structures in surfactant solutions: liquid crystals, both thermotropic and lyotropic, polymer solutions, gels, and melts: colloidal suspensions, including ferrofluids, electro-rheological fluids, etc.

Biologically inspired physics. Models in evolutionary biology and population genetics, evolution of complexity, neural networks, optimization, protein folding, motor proteins and molecular ratchets, etc.

Qualified researchers interested in submitting project proposals to be considered for funding may write to the following address for further details:

The Convener (Dr Praveer Asthana)
PAC on Nonlinear, Complex and Biological Systems
SERC Division
Department of Science & Technology
Technology Bhavan
New Mehrauli Road
New Delhi 110 016

Government of India
MINISTRY OF SCIENCE & TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

AWARD OF BIOTECHNOLOGY OVERSEAS ASSOCIATESHIP: 1996-97

Applications are invited from Indian Nationals for the award of Biotechnology Overseas Associateships (1996-97) for conducting advanced research or undergoing specialized research training in overseas research institutes/laboratories in the priority areas of biotechnology, viz. (i) Basic molecular biology; (ii) Microbial genetics; (iii) Recombinant DNA technology; (iv) Molecular immunology, immunogenetics and immunodiagnostics; (v) Molecular virology; (vi) Animal tissue culture, human hybridomas and cell-culture-based vaccines; (vii) Plant tissue-culture; (viii) Genetic disorders and gene therapy; (ix) Animal biotechnology; (x) Aquaculture and marine biotechnology; (xi) Peptide and nucleic acid chemistry and applications; (xii) Protein and enzyme engineering; (xiii) Modern techniques in industrial microbiology and bio-conversion; (xiv) Biochemical engineering, bio-separation, process optimization and computer modelling; (xv) Bio-informatics; (xvi) Emerging areas.

The applicants should possess a Ph D/M D degree in the area related to biotechnology, with a good record of research contributions in biotechnology. The candidates should hold a regular/permanent position in research institutions/universities and should be actively engaged in biotechnological R&D work. Applications from persons working on temporary positions such as research fellows or associates will not be considered.

Details of the Award

1) Two categories of Associateships, namely Long-term and Short-term associateships are awarded. 2) The Long-term Associateship is awarded for a period of one year only and it is not extendable. The Short-term Associateship is normally awarded for three months only. However, the Short-term Associateship may be awarded for a maximum period up to six months in cases where a longer duration is necessary and justified. Such proposals will be considered by the Department purely on the merit of the case and the decision of the Department will be final. 3) The Long-term Associates are entitled to (a) Monthly Associateship @ US \$1500 or its equivalent in the country of study; (b) Personal Equipment Grant of Rs 4000 (one time grant); (c) Cost of air passage in economy class by Air India for joining overseas laboratory and back; 4) The Short-term Associates are entitled to (a) Monthly Associateship @ US \$1800 or its equivalent in the country of study; (b) Cost of air passage for joining overseas laboratory and back. 5) The selected candidates may receive the payment of salary and continuance of other service benefits by the institutions to which they belong. However, no liability on any of this account will be borne by DBT; 6) Selected candidates shall be required to execute a Service Bond to serve in India for a period of 1 to 3 years depending upon the duration/category of the Associateship awarded after return to India. Candidates should submit their applications typed on plain paper (one copy) complete with enclosures in the format given below *Through Proper Channel* to the Sr. Scientific Officer-I (Attention: Mrs. Suchita Ninawe), Department of Biotechnology, Block-II, 6th Floor, CGO Complex, Lodhi Road, New Delhi 110 003 on or before *15th January 1997*.

Age Limit

- (a) Long-term Associateship: not exceeding 40 years.
- (b) Short-term Associateship: not exceeding 50 years.

Candidates *should not have crossed* the above prescribed age limit as on 15th January 1997, the last date for receipt of application. The above prescribed age limits will not be relaxed.

FORMAT

Application for the Biotechnology Overseas Associateship 1996-97

1) Category of the Associateship Applied for (Long-term or Short-term); 2) *In the case of Short-term Associateship only*, (a) Duration of the Associateship applied for, (b) Justification (in case the proposed duration exceeds three months); 3) Applicant's name (in full), designation and address; 4) Father's/husband's name (in full); 5) Date, place of birth and age (as on 15th January 1997); 6) Academic qualifications (degree onwards with year, name of the institution, subjects of specialization and distinctions, if any); 7) Details of the nature and duration of the present and past employment; 8) Particulars of current research work (not exceeding one page); 9) Research publications in indexed journals during the last five years (enclose a list; exclude presentations in seminars/symposia; attach reprints of 3 research papers which you consider the best); 10) Details of the proposed subject of research/training and its relevance to the priority areas identified by DBT and the plans and programmes of the parent institute (the proposal should be concise, the objectives should be clearly stated and it should not exceed 2 pages); 11) Proposed place of research/training (indicate whether the consent of host institutions has been obtained); 12) Details of past overseas visits (mention the country visited, purpose, year and duration); 13) Have you been previously selected for any Associateship award (National/Overseas) of DBT? If so, give details; 14) Particulars of R&D projects sanctioned by DBT (ongoing/completed), if any; 15) Priority area of the proposed research/training.

Place

Date

Signature of the Candidate

Statement from the Present Employer

As per the terms and conditions of the Associateship, the candidates selected for the awards should be granted deputation terms like payment of salary and continuation of other service benefits during the Associateship by the employers. The employer's statement should contain a certificate to this effect as also about continuity of employment after the Associateship period. Applications which are forwarded without the employer's statement may not be considered.

NOTE: 1) The candidates should correspond themselves with their proposed host institution abroad for placement; 2) Only applications sponsored by the employer and forwarded by the head of the institution/organization shall be considered for selection for the award of the Associateships. The applicants are therefore, advised to ensure that their applications *Through Proper Channel* should reach the Department on or before the closing date; 3) All the applicants will be informed about the result of selection in due course and therefore, no interim queries in this regard will be attended to.

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